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Glutathionylation of proteins by glutathione disulfide S-oxide

Kuo-Ping Huang*, Freesia L. Huang

Section on Metabolic Regulation, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Building 49, Room 6A36, 49 Convent Drive MSC 4510, Bethesda, MD 20892-4510, USA

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Abstract

Aqueous solution of S-nitrosoglutathione (GSNO) underwent spontaneous chemical transformation that generated several glutathione derivatives including glutathione sulfonic acid (GSO_3H), glutathione disulfide S-oxide (GS(O)SG), glutathione disulfide S-dioxide, and glutathione disulfide. Surprisingly, GS(O)SG (also called glutathione thiosulfinate), which was not identified as a metabolite of GSNO previously, was one of the major products derived from GSNO. This compound was very reactive toward any thiol and the reaction product was a mixed disulfide. The rate of reaction of GS(O)SG with 5-mercapto-2-nitro-benzoate was nearly 20-fold faster than that of GSNO. The mechanism for the formation of GS(O)SG was believed to involve the sulfenic acid (GSOH) and thiosulfinamide ($GS(O)NH_2$) intermediates; the former underwent self-condensation and the latter reacted with GSH to form GS(O)SG. Many reactive oxygen and nitrogen species were also capable of oxidizing GSH or GSSG to form GS(O)SG, which likely played a central role in integrating both the oxidative and nitrosative cellular responses through thionylation of thiols. Treatments of rat brain tissue slices with oxidants resulted in an enhanced thionylation of proteins with a concomitant increase in cellular level of GS(O)SG, suggesting that this compound might play a second messenger role for stimuli that produced a variety of oxidative species.

Keywords: Glutathione disulfide S-oxide (glutathione thiosulfinate); Glutathionylation; Neurogranin; Oxidative and nitrosative stress; S-Nitrosoglutathione; Signal transduction

Redox regulation through modifications of proteins has emerged as one of the major cellular responses to oxidative and nitrosative stresses [1–4]. A wide range of modifications of amino acids in protein, including formation of S-nitrosocysteine, cysteine sulfenic acid (Cys-SOH), sulfinic acid ($Cys\text{-}SO_2H$), sulfonic acid ($Cys\text{-}SO_3H$), inter- and intramolecular disulfide, and mixed disulfide with glutathione (GSH) (Cys-S-SG), methionine sulfoxide and sulfone, carbonyl formation at lysine and arginine, dityrosine formation, and nitration of tryptophan and tyrosine have been identified [4–7]. Many of these modifications are potential sensors of the redox states to changing environment induced by growth factor [8–10], hormone [11], neurotransmitter [12], and cytokine [13]. Thionylation of protein is one of the mechanisms that can serve as a protection against the oxidative insult as well as for cell signaling. Protein S-glutathionylation introduces the γ-glutamyl tripeptide with additional ionic charges into a protein resembling the well-characterized mechanism of protein phosphorylation in cellular regulation. The potential target proteins for thionylation are likely as abundant as those for phosphorylation. However, unlike protein phosphorylation where numerous protein kinases have been identified, there is no evidence for the involvement of specific thionylating enzyme for each target protein [1], although it has been suggested that thioltransferases may function in both thionylation and dethionylation reactions [14]. Thus, the specificity for the thionylation may be endowed within each protein depending on its affinity for the modifiers, namely, thionylating agent, and the accessibility of the sulfhydryl group. As for the thionylating agent,

* Corresponding author. Tel.: +1-301-496-7827; fax: +1-301-496-7434.
E-mail address: kphuang@helix.nih.gov (K.-P. Huang).

Abbreviations: GSNO, S-nitrosoglutathione; GSH, glutathione; GSO_3H, glutathione sulfonic acid; GS(O)SG, glutathione disulfide S-oxide; $GS(O)_2SG$, glutathione disulfide S-dioxide; GSSG, glutathione disulfide; GSOH, glutathione sulfenic acid; Cys-SOH, cysteine sulfenic acid; $Cys\text{-}SO_2H$, cysteine sulfinic acid; $Cys\text{-}SO_3H$, cysteine sulfonic acid; Cys-S-SG, cysteine-glutathione mixed disulfide; NO, nitric oxide; RSNO, S-nitrosothiol; RONO, alkyl nitrite; $GS^{\bullet}$, glutathione thiyl radical; $GSOO^{\bullet}$, glutathione peroxy radical; $O_2^{\bullet -}$, superoxide; GS-NH-SG, glutathione thiosulfenamide; HNO, nitroxyl; GSNHOH, glutathione *N*-hydroxysulfenamide; Cys-S(O)S-Cys, cystine-S-oxide; L-S(O)S-L, lipoic acid thiosulfinate; $L\text{-}S(O)_2S\text{-}L$, lipoic acid thiosulfonate; Ng, neurogranin/RC3; MNB, 5-mercapto-2-nitro-benzoate.

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it has to be highly reactive and preferably having a short half-life, so that the reaction will be localized nearby the origin of the oxidant. Recently, we have identified a highly reactive glutathionylating agent, GS(O)SG, from the aqueous solution of GSNO, which may fulfill some of these features [15]. This compound, also called glutathione thiosulfinate, is the anhydride of glutathione sulfenic acid (GSOH). Introduction of an oxygen atom into a disulfide bond significantly decreases the bond energy and transforms it into a highly reactive agent toward thiol [16].

1. Synthesis and decomposition of GSNO

Since the discovery of nitric oxide (NO) as a messenger for a variety of cellular functions, considerable interest has been focused on the biological chemistry and clinical potential of S-nitrosothiol (RSNO), which serve as a reservoir of the short-lived NO [17–19]. GSNO is one of the major endogenous metabolites of NO that have been detected in both extra- and intra-cellular spaces and is believed to mediate some of the actions of NO [20]. GSNO can be synthesized by treatment of GSH with nitrosating reagents at a stoichiometric ratio of 1:1 under mild acidic condition [21]. A variety of reagents, including nitrous acid, dinitrogen tetroxide, dinitrogen trioxide, nitrogen dioxide, nitrosyl chloride, and alkyl nitrite (RONO), are effective in nitrosation of GSH. In principle, any reagent acting as a carrier of NO^+ is sufficient. In biological system, GSNO is formed primarily by nitrosation of GSH with NO generated by NO synthase in the presence of oxygen, which produces N_2O_3 as electrophilic nitrosating agent.

GSNO decomposes upon heating to form glutathione disulfide (GSSG) and NO, which is then oxidized by oxygen to form NO_2 [18]. Similar reaction occurs photochemically to give NO and thiyl ($GS^\bullet$) radical, which reacts with GSNO to give GSSG and additional NO or with oxygen to give the peroxy radical ($GSOO^\bullet$). $GSOO^\bullet$ also reacts with GSNO to form GSSG and NO [18,22]. GSNO also reacts with superoxide ($O_2^{\bullet -}$) to form peroxynitrite and thiyl radical by a mechanism involving $O_2^{\bullet -}$-dependent reduction of GSNO to yield NO, which in turn reacts with a second $O_2^{\bullet -}$ to yield peroxynitrite [23]. In aqueous solution at physiological pH, GSNO, as compared to CysSNO, is relatively stable in the presence of metal chelator; however, it decomposes rapidly in the presence of copper ions [24]. It was demonstrated that any reducing agent capable of reducing Cu^{2+} to Cu^+ is suffice to trigger decomposition of GSNO to release NO and to give GSSG [25]. GSNO undergoes transnitrosation with other thiol to form GSH and another RSNO. In addition, thiols can trigger a complex set of chain reactions, which involve initial formation of glutathione *N*-hydroxysulfenamide (GSNHOH) or nitroxyl (HNO) [26,27]. Several products, including NO, ammonia, nitrous oxide (N_2O), nitrite (NO_2^-), hydroxylamine, and GSSG have been identified. *In vivo*, GSNO can be metabolized by a reductase, glutathione-dependent formaldehyde dehydrogenase, which utilizes NADH to reduce GSNO to *N*-hydroxysulfenamide (GSNHOH) and $GSNH_2$, the latter reacts with GSH to form GSSG and NH_3 [28].

2. Isolation of the glutathione derivatives from the decomposed GSNO

Much of the studies on the reactions of GSNO have been focused on the identification of nitrogen-related products and it is generally believed that the end product of glutathione moiety is GSSG. Although the existence of other oxidized forms of GSH have been proposed as intermediates [26,27], none of them have been positively identified, perhaps, due to their instability. Spontaneous decomposition of GSNO in aqueous solution at mild acidic pH proceeds slowly, it allows the accumulation of the acid-stable intermediates for identification by HPLC and ES/MS [15]. Several glutathione derivatives have been identified from the partially decomposed GSNO (mass 337 Da), including GSO_3H (mass 356 Da), stereoisomers of GS(O)SG (mass 629 Da), glutathione disulfide S-dioxide ($GS(O)_2SG$, mass 645 Da), and GSSG (mass 613 Da). When tested for their efficacies to oxidize Cys residues in rat brain neurogranin (Ng), both stereoisomers of GS(O)SG were the most potent among them. While GSO_3H, $GS(O)_2SG$, GSNO, and GSSG were also effective in causing rat brain Ng oxidation to form intramolecular disulfide, they produced very little glutathionylation. GS(O)SG, on the other hand, caused extensive glutathionylation of this protein [15].

3. Synthesis of GS(O)SG by oxidation of disulfide or thiol with peracids and peroxides

The existence of S-monoxide (thiosulfinate) of cysteine was suggested in the 1930s and the alkyl thiosulfinate was first synthesized in the 1940s by oxidation of a disulfide with one mole of peracid [29]. In 1964, Savige *et al.* reported the synthesis of S-monoxide of cystine by oxidation with peracetic acid (CH_3CO_3H) or performic acid (HCO_3H) under acidic conditions [30]. L-Cystine (±)-S-oxides (Cys-S(O)S-Cys) were obtained as a diastereoisomeric mixture. Cystamine S-oxide was also prepared by oxidation of cystamine with hydrogen peroxide at pH 0–5 in the absence of halide. DL-Homocystine-S-oxide and -S-dioxide were obtained by oxidation with peracetic acid in the absence and presence of hydrochloric acid, respectively. In addition, dialkyl disulfides can also be photooxidized to S-oxide without further oxidation to higher oxide [31]. Thiosulfinates are susceptible to nucleophilic attack at S(O)-sulfur or S-sulfur with cleavage of sulfur–sulfur bond. Chloride, bromide, and iodide have been

shown to promote the conversion of the disulfide S-oxide to disulfide and disulfide S-dioxide. In 1981, Finley *et al.* [32] showed that oxidation of GSH with several oxidants, including hydrogen peroxide (H_2O_2), benzoyl peroxide [$(C_6H_5CO)_2O_2$], potassium bromate ($KBrO_3$), and linoleic acid hydroperoxide, generated GSSG, GS(O)SG, $GS(O)_2$-SG, GSO_2H, and GSO_3H. The rate of oxidation increased with increasing pH and the S-oxide and S-dioxide were the major products when GSH was oxidized by equimolar of H_2O_2. When lipid hydroperoxide was used as the oxidant, the proportion of (monoxide + dioxide)/GSSG was much greater than that caused by oxidation with H_2O_2 [32]. Oxidation of organic disulfide by H_2O_2 to give RS(O)SR in high yield can be achieved in the presence of catalyst methyltrioxorhenium(VII) [33]. After prolonged reaction period, $RS(O)_2SR$ and RSO_3H are also formed. Formation of lipoic acid thiosulfinate (L-S(O)S-L) and thiosulfonate ($L\text{-}S(O)_2S\text{-}L$) by oxidation with neutrophile oxidant, hypochlorous acid (HOCl), have been reported [34]. Information regarding the reaction involving GS(O)SG is rather limited due to its instability. However, certain properties of GS(O)SG can be inferred from the previous work of Savige *et al.* on Cys-S(O)S-Cys [30]. This compound is easily hydrolyzed in aqueous solution and reacts rapidly with two molar proportions of certain thiol to give almost exclusively mixed disulfide:

$$3\text{Cys-S(O)S-Cys} + H_2O \rightarrow 2\text{Cys-SS-Cys} + 2\text{Cys-SO}_2H$$

$$\text{Cys-S(O)S-Cys} + 2\text{RSH} \rightarrow 2\text{Cys-SS-R} + H_2O$$

Calam and Waley [35] reported that $GS(O)_2SG$ also reacted with thiol to form mixed disulfide and GSO_2H:

$$GS(O)_2SG + RSH \rightarrow GSSR + GSO_2H$$

4. Generation of GS(O)SG from GSNO

The detailed mechanism for the generation of GS(O)SG has not yet been worked out. We speculate that the initiation of GSNO decomposition is likely catalyzed by copper ions or by homolysis that yields GSH and NO [18].

$$Cu^+ + GSNO + H^+ \rightarrow Cu^{2+} + GSH + NO$$

GSH then reacts with GSNO forming adduct *N*-hydroxysulfenamide (GS-N(OH)-SG) [26], which rearranges to form sulfinamide (GS(O)-NH-SG).

$$GSNO + GSH \rightarrow \text{GS-N(OH)-SG} \leftrightarrow \text{GS(O)-NH-SG}$$

The glutathione thiosulfinamide can subsequently react with GSH to form GS(O)SG and $GSNH_2$ or GS-NH-SG and GSOH.

$$\text{GS(O)-NH-SG} + GSH \rightarrow GS(O)SG + \text{GS-NH}_2$$

or

$$\text{GS(O)-NH-SG} + GSH \rightarrow GS(O)NH_2 + GSSG$$

$$GS(O)NH_2 + GSH \rightarrow GS(O)SG + NH_3$$

Alternatively, GSNO can react with GSH to form GSSG and HNO and HNO in turn reacts with GSH to give *N*-hydroxysulfenamide, which rearrange to generate sulfinamide [27].

$$GSNO + GSH \rightarrow GSSG + HNO$$

$$GSH + HNO \rightarrow \text{GS-NH-OH} \leftrightarrow GS(O)NH_2$$

Reaction of $GS(O)NH_2$ with GSH forms GS(O)SG and NH_3 and with water to form sulfinic acid and NH_3, whereas reaction of GS-NH-OH with GSH forms GSSG and NH_2OH.

$$GS(O)NH_2 + GSH \rightarrow GS(O)SG + NH_3$$

$$GS(O)NH_2 + H_2O \rightarrow GS(O)OH + NH_3$$

$$\text{GS-NH-OH} + GSH \rightarrow GSSG + NH_2OH$$

Further oxidation of GS(O)SG and GS(O)OH generate $GS(O)_2SG$ and sulfonic acid (GSO_3H), respectively. The key intermediates leading to the synthesis of GS(O)SG are the sulfinamides ($GS(O)NH_2$ and GS(O)-NH-SG) proposed in the two previous studies [26,27], although neither found GS(O)SG as a reaction product. This can be explained by the high reactivity of this compound with thiol, which was used to trigger the rapid decomposition of GSNO. We found that even at pH lower than 3 (in 0.1% trifluoroacetic acid), GS(O)SG can still react with GSH to form GSSG. Although GS(O)SG is relatively stable at acidic pH in the absence of thiol, it hydrolyzes to give GSSG at neutral pH. The proposed schemes seem to satisfy the generation of the various decomposition products of GSNO seen in our work with the exception of sulfinic acid [15], which is probably oxidized by NO or higher nitrogen oxides (NO_x) to form sulfonic acid. Obviously, other mechanisms are still possible. Since homolytic decomposition of GSNO generates thiyl radical, which can react with O_2 to form glutathione peroxysulfenyl radical ($GSOO^\bullet$) and GSOOH. GSOOH can be reduced by GSH to form GSOH or it can oxidize GSSG to form GS(O)SG. GSOH is unstable and may self-condense to form GS(O)SG, analogous to that for the synthesis of alk(en)yl thiosulfinates from their corresponding sulfenic acids [36]. All the above-described reactions for GSNO are likely to occur for the S-nitroso compounds of cysteine, homocysteine, and *N*-actylpenicillamine.

5. S-Glutathionylation of proteins by GS(O)SG

Two rat brain proteins, neurogranin/RC3 (Ng) and neuromodulin/GAP-43 (Nm), have been tested for glutathionylation by GS(O)SG [15]; the former contains four and the latter two Cys residues. Ng and Nm are two neuronal Ca^{2+}-sensitive calmodulin-binding proteins, whose phosphorylations by protein kinase C attenuate their binding affinities for calmodulin [37,38]. Oxidation of Ng by a variety of oxidants generates mostly intramolecular

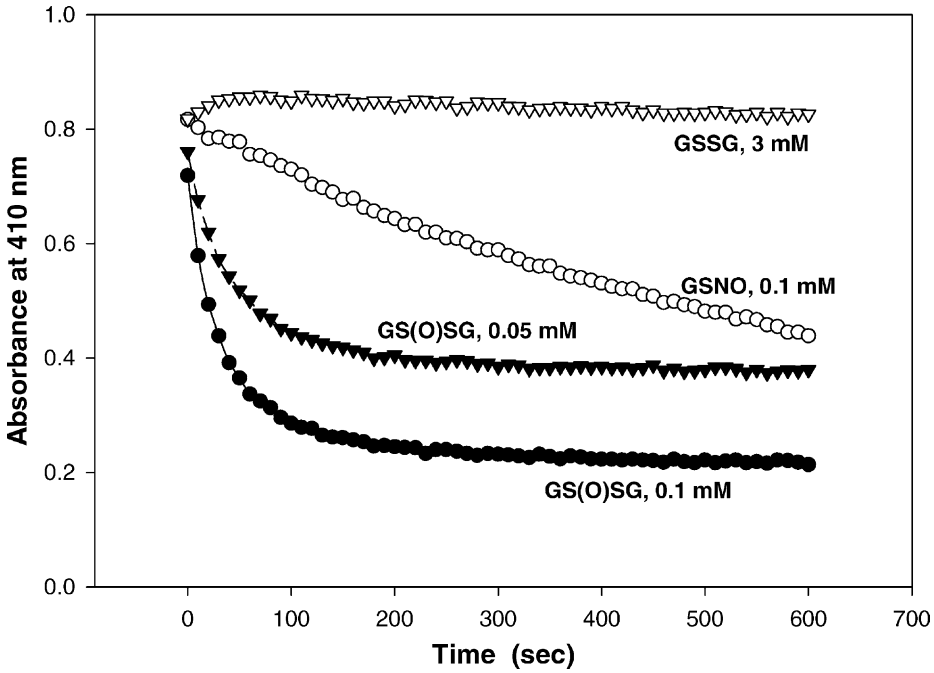

Fig. 1. Oxidation of 5-mercapto-2-nitro-benzoate by GSNO and GS(O)SG. The rates of oxidation of 5-mercapto-2-nitro-benzoate (0.24 mM) by GSNO (0.1 mM), GS(O)SG (0.05 and 0.1 mM), and 3 mM GSSG were measured spectrophotometrically at 410 nm. The reaction rate with 0.1 mM GS(O)SG is approximately 20-fold greater than that of the same concentration of GSNO. At 3 mM, GSSG was not effective in oxidizing 5-mercapto-2-nitro-benzoate.

disulfide bonds, which transform the protein into a compact form distinguishable from the reduced form by non-reducing SDS–PAGE [39–41]. Oxidation of Ng to form intramolecular disulfides also resulted in an attenuation of the binding affinity for calmodulin [42]. Treatment of Ng with freshly prepared GSNO generated intramolecular disulfide presumably mediated initially by transnitrosation and followed by intramolecular disulfide formation. In contrast, treatment of this protein with GS(O)SG produced nearly all glutathionylation [15]. This phenomenon can be explained by the relative rates of oxidation of 5-mercapto-2-nitro-benzoate (MNB), derived from the reduction of Ellman reagent (5,5′-dithiobis(2-nitrobenzoic acid), by GS(O)SG and GSNO (Fig. 1). Oxidation of MNB, measured spectrophotometrically by the reduction of absorbance at 410 nm, by GS(O)SG was at least 20 times faster than that by GSNO. Apparently, all the –SH groups within the Ng molecule are accessible to GS(O)SG and become rapidly thionylated, whereas nitrosation of Ng by GSNO is a slower reaction and thus allows intramolecular disulfide formation. In principle, both sulfur centers of GS(O)SG are susceptible to nucleophilic attack by thiolate anion to form Ng-S-SG and Ng-S-S(O)G; however, the product identified by ES/MS contains only the former. This finding indicate that Ng-S-S(O)G is also unstable and is subjected to further hydrolysis or reduction by GSH.

$$\text{Ng-SH} + \text{GS(O)SG} \rightarrow \text{Ng-S-SG} + \text{GSOH}$$

or

$$\text{Ng-SH} + \text{GS(O)SG} \rightarrow \text{Ng-S-S(O)G} + \text{GSH}$$

Treatment of Nm with GS(O)SG also resulted in a complete glutathionylation of both –SH groups. A recent report showed that treatment of a synthetic peptide corresponding to the autoinhibitory domain of the precursors of matrix metalloproteinase with S-nitroglutathione ($GSNO_2$) produced a stable thionylated product with a predicted structure of disulfide S-oxide, which is resistant to dethionylation by dithiothreitol [43]. The unusual stability of this product could be due to protection of the thionylation site by the amino acid side-chains surrounding the modified Cys residue.

The stoichiometry and rate of GS(O)SG-mediated modification of proteins containing multiple Cys residues are difficult to monitor due to the competing reactions to form protein GSH mixed disulfide and inter- and intramolecular disulfides. Treatment of Cys with GS(O)SG at pH 7.2 results in a nearly instantaneous formation of mixed disulfide with a stoichiometry of Cys/GS(O)SG = 2, suggesting that both GS-moiety are utilized according to the following reaction:

$$2\text{Cys-SH} + \text{GS(O)SG} \rightarrow 2\text{CysS-SG} + \text{H}_2\text{O}$$

Thionylation of Cys can even take place in 0.1% trifluoroacetic acid (pH less than 3), although at a lower stoichiometry.

6. Oxidant-mediated thionylation of proteins

Glutathionylation of proteins is a potential mechanism for cell to transduce the oxidative and nitrosative signals

into functional responses. Several recent reviews [1–4] have discussed the potential mechanisms for the oxidant-mediated protein glutathionylation including the following: (1) thiol-disulfide exchange between protein thiols and GSSG [44]; (2) oxidation of protein thiols by oxyradicals or H_2O_2 to form thiyl radicals or sulfenic acids and then interacts with GSH to produce mixed disulfide [45]; (3) nucleophilic attack of protein thiolate on GSNO to produce mixed disulfide [46–49]; (4) oxidation of GSH to form sulfenic acid and then interacts with protein thiols to form mixed disulfides [47]; and (5) nitrosation of protein thiols followed by interaction with GSH to form mixed disulfides [46,47]. With the discovery of GS(O)SG as a potent glutathionylating agent, it is pertinent to determine if this compound is functioning *in vivo*. We took the approach of correlating the glutathionylation of proteins with an increase in cellular content of GS(O)SG. However, one should bear in mind that GS(O)SG is a very reactive compound and its steady state level will be low. It is essential to freeze the treated samples immediately, block all the sulfhydryl groups in the homogenates, and lower the pH to below 4 to maintain the stability of this compound for further analysis. Even with these precautions the estimated level of GS(O)SG will probably be underestimated. Indeed, treatment of rat brain slices with several oxidants caused an increase in both stereoisomers of GS(O)SG based on the elution profile of HPLC and concomitant increase in protein thionylation [15]. Thionylation of Ng was positively identified by immunoprecipitation and Western blot analysis.

The question remains as whether GS(O)SG derived from GSNO or from other sources is responsible for protein thionylation *in vivo*. There is no easy answer to this question and the best guess is that there are multiple sources for GS(O)SG formation. GSNO can be generated by the action of many NO metabolites [50] and it can serve as a precursor of GS(O)SG through GSH-mediated decomposition or the GSNO reductase-catalyzed reduction to generate GSNHOH [28], followed by rearrangement to form $GS(O)NH_2$ [27], and subsequent reaction with GSH to form GS(O)SG. It can also be formed by the reaction of GSH with HNO, which has a predicted pK_a of 7.2 ± 1.0 [51], to give the same initial product as the GSNO reductase-catalyzed reaction.

$$GSH + HNO \rightarrow GSNHOH \leftrightarrow GS^{+} = NH \leftrightarrow GS(O)NH_2$$

$$GSH + GS(O)NH_2 \rightarrow GS(O)SG + NH_3$$

HNO or nitroxyl anion (NO^{-}), the one electron reduction product of NO, can be generated by reduction of NO by superoxide dismutase [52], reduction of NO by mitochondrial cytochrome *c* [53], and interaction with ubiquinol [54]. HNO is also a product of NO synthase decoupled from tetrahydrobiopterin [55]. Other possibilities of forming GS(O)SG are: direct oxidation of GSH by superoxide, H_2O_2 [56], peroxynitrite [57], hypochlorous acid (HOCl) [34], and lipid hydroperoxides [32] to form sulfenic acid (GSOH) and followed by condensation to form GS(O)SG. Sulfenic acids (RS-OH) in general are very reactive and usually are not detected because they formed hydrogen-

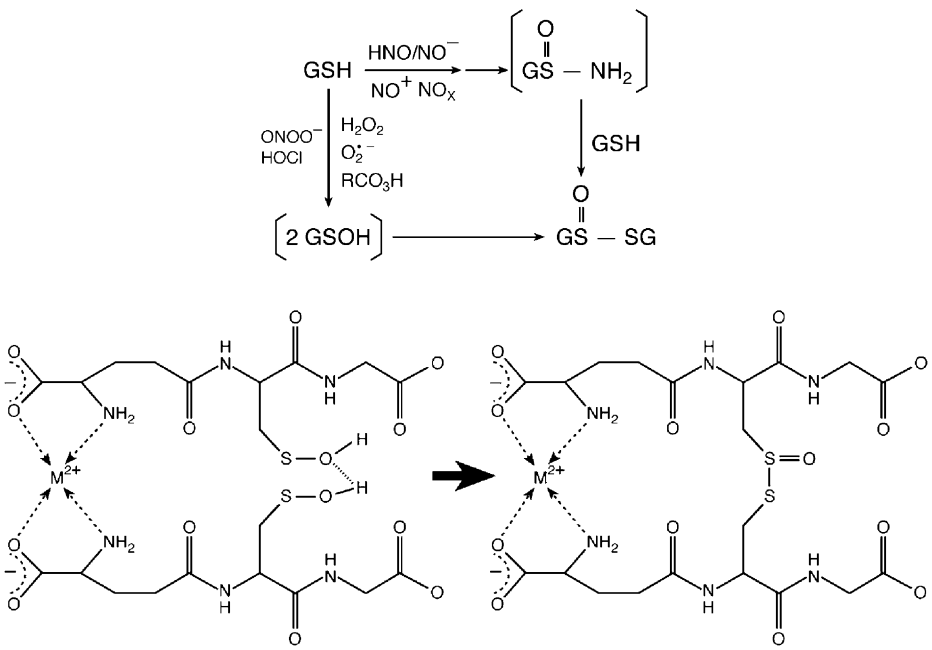

Fig. 2. Reactive oxygen- and nitrogen-species mediated generation of GS(O)SG. Many reactive oxygen and nitrogen species are capable of oxidizing GSH to form sulfenic acid (GSOH) or sulfinamide ($GS(O)NH_2$), the former self-condenses and the latter reacts with GSH to form GS(O)SG. GSOH may form dimer, which is stabilized by binding of divalent metal ion with two glutamate residues and hydrogen bond between the –OH groups. This structure favors the dehydration of two GSOH to form GS(O)SG. Similarly, the dimer consists of $GS(O)NH_2$ and GSH will favor the condensation to form GS(O)SG.

bonded dimer, which self-condensed to form thiosulfinates (RS(O)SR) [36,58]. This condensation process is unique, as compared to carboxylic acid, in the fact that the anhydride (RS(O)SR) is strongly preferred thermodynamically [59]. We propose that the condensation of GSOH to form GS(O)SG is probably in the context of dimer maintained by both hydrogen bond and divalent metal ion chelation with glutamate (Fig. 2). The formation of GSSG and Cu^{2+} complexes have been proposed by Noble and Williams [60]. A direct oxidation of GSSG by microsomal cytochrome P-450 and flavin-containing monooxygenases is also a possibility to form GS(O)SG as that shown for the oxidation of diallyl disulfide to form allicin [61].

7. Conclusion

Significant progress has been made regarding the molecular basis of redox sensitivity in the modifications of proteins; however, the *in vivo* mechanism for these modifications is far from completely understood. Thionylation and dethionylation of protein are important mechanisms for cellular protection as well as for signal transduction for a variety of stimuli, which induce changes in intracellular redox potential and formation of reactive oxygen and nitrogen species. These mechanisms transform the potent oxidants into reversible forms of protein modifications, which can be reversed when the oxidants dissipate. Several proteins involved in the regulation of signaling mechanisms for cellular metabolism, cell cycle progression, gene expression, and apoptosis have been identified to be thionylated. The state of protein thionylation/dethionylation is likely maintained in a rapid dynamic equilibrium depending on the cellular redox state. To fulfill this requirement, it is necessary to have a highly reactive modifier to mediate the various responses. We propose that GSH, cysteine, as well as other sulfhydryl-containing cellular compounds and drugs may serve as targets of oxidants to generate disulfide S-oxides, which in turn modify protein thiols. These compounds can potentially be generated from those oxidants capable of oxidizing sulfhydryl group to form sulfenic acid or sulfinamides, including NO^+, HNO/NO^-, NO_x, GSNO, superoxide, peroxynitrite, hydroxy radical, H_2O_2, and lipid hydroperoxide. Oxidation of GSSG by microsomal oxygenases, peracids, peroxides, and hypochlorous acid may also lead to formation of GS(O)SG. Based on these predictions we surmise that the most abundant source of disulfide S-oxide, GS(O)SG, plays a central role in the metabolism of GSH involving disulfide formation with any sulfhydryl compound (Fig. 3). The high reactivity of GS(O)SG is especially useful for the localized dynamic response in the nervous system, where the formation of this compound can coincide with the pulse of the neurotransmitter-mediated activation of NO synthases or production of superoxide [62] at the targeted synapases.

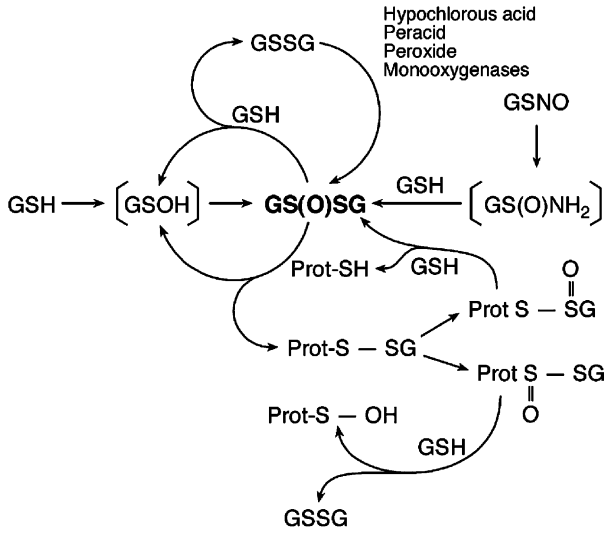

Fig. 3. Hypothetical role of GS(O)SG in glutathione metabolism. The cellular level of GS(O)SG will be fluctuated depending on the redox state and serves as a driving force to generate GSSG or mixed disulfide with proteins or other thiols. GSOH generated by direct oxidation of GSH or by reaction of GS(O)SG with GSH will self-condense to continue supply GS(O)SG as long as the oxidants are present. GSNO can also form GS(O)SG through sulfinamide intermediate. GSSG can be oxidized directly by oxidants or monooxygenases to form GS(O)SG. Protein-S-SG can also be oxidized to generate disulfide S-oxide, which further reacts with GSH to form either GS(O)SG or protein sulfenic acid.

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