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Forum Review

Cellular and Molecular Targets of Protein *S*-Glutathiolation

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ABSTRACT

Oxidative stress and reactive oxygen species play a major role in both normal and pathophysiologic cellular processes. Although many cellular constituents can be damaged by oxidant exposure, cysteine thiol groups are among the most readily oxidized moieties found within cells. To avoid potentially irreversible cysteine thiol oxidation, cells have developed multiple antioxidant defenses to preserve these moieties. Among these defenses, protein *S*-glutathiolation has emerged as an important mechanism, both in the maintenance of thiol stability during oxidant exposure and as a rapid and efficient mechanism regulating protein activity and cellular metabolic pathways. Here we review the known molecular targets of *S*-glutathiolation, with emphasis on the varying molecular effects of *S*-glutathiolation on different proteins. *Antioxid. Redox Signal.* 7, 940–950.

INTRODUCTION

OVER THE PAST THREE DECADES, the interaction of reactive oxygen species (ROS) with cellular components has become increasingly important in the study of both normal and pathological processes. At low concentrations, ROS function in many normal processes. For example, hydrogen peroxide (H_2O_2), nitric oxide ($^{\bullet}NO$), and possibly superoxide ($O_2^{-\bullet}$) function in signal transduction pathways under normal physiologic conditions (45, 54). Additionally, ROS regulate immune activity and host defense, cell proliferation, differentiation, and death, and the aging process (4, 6, 66, 104). At higher concentrations (*i.e.,* oxidative stress), ROS may damage nearly all biomolecules, including nucleic acids, proteins, lipids, and sugars—a state associated with a vast array of diseases, including neoplastic, autoimmune, neurodegenerative, and cardiovascular disease (10, 13, 17, 28, 44, 46, 47, 53, 69, 76, 88, 156, 160, 165).

ROS species are produced by multiple processes, including normal mitochondrial activity, ionizing and ultraviolet radiation exposure, exogenous and endogenous compound metabolism, growth factor signal transduction pathways secondary to receptor ligation, and pathological metabolic processes, such as seen in hemochromatosis and neurodegenerative diseases (5, 64, 76, 127). Under normal conditions, the greatest ROS source is normal mitochondrial respiratory activity (154), which converts roughly 1–3% of the oxygen used in oxidative phosphorylation into $O_2^{-\bullet}$ (16, 153). Mitochondrial $O_2^{-\bullet}$ is converted either spontaneously or by manganese superoxide dismutase into H_2O_2, which diffuses into the extramitochondrial space where it may exert prooxidant effects (see below; 48). Other common ROS include many partially reduced oxygen and oxygen/nitrogen species such as H_2O_2, the hydroxyl radical ($^{\bullet}OH$), hypochlorous acid, singlet oxygen ($^1O_2$), the peroxynitrite anion ($ONOO^-$) and $^{\bullet}NO$ (44). As some of these species contain nitrogen, the term "nitrosative stress" is used to describe the effect of excessive/dysregulated $^{\bullet}NO$ and $^{\bullet}NO$-derived reactive nitrogen species (RNS) on the cellular redox states (67).

Due to the toxic nature of many ROS/RNS, it is not surprising that aerobic organisms have elaborate oxidant defense mechanisms. These defenses include enzymatic and chemical antioxidants, precise P_{O_2} regulation, transition metal chelation, and alterations in gene-regulatory pathways to ameliorate/lessen oxidant toxicity (63, 68, 69, 86, 119, 135, 136). The specific cellular responses to oxidative stress appear to depend on the severity of the stress, with low-level oxidative stress often inducing proliferative responses in mitotically competent cells, checkpoint responses/growth arrest and/or differentiation at higher ROS concentrations, and

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apoptosis and cell death at high ROS concentrations (66, 131). Last, a significant portion of ROS-mediated cell damage involves the conversion of less reactive ROS into more toxic species, often involving a transition metal as a catalyst. The generation of the highly reactive •OH radical produced by the interaction of H_2O_2 with labile Fe^{2+} via Fenton chemistry is an example of such an event (152). Thus, intra- and extracellular chelation of transition metals such as iron and copper plays a significant role in cellular antioxidant defenses (31, 118).

OXIDANTS AND CYSTEINE THIOL GROUPS

Whereas ROS such as •OH readily damage nearly all cellular constituents, other ROS/RNS, such as H_2O_2, $O_2^{-\bullet}$, and •NO, have significantly lower reactivities. •NO, in fact, can exert antioxidant effects via chain-breaking free radical scavenging (130). Due to their low reactivity, these oxidants are thought to react mainly with easily oxidized cellular components. Under physiological conditions, thiol moieties, such as those found in the cysteine side chain, are the most chemically reactive sites on proteins (30). Additionally, sulfur has roughly 10 oxidation states within cells, ranging from −2 in sulfides to +6 in heparin sulfate. Thus, sulfur redox chemistry lends itself to quite complex biochemical activity (51). Recently, considerable data have accumulated indicating that cysteine thiol redox reactions play an important role in antioxidant defense, the regulation of protein function, and oxidant-mediated signal transduction. Here we will review cysteine thiol *S*-glutathiolation following oxidative/nitrosative stress and its effect on protein function and the activity of several transcription factors.

Cysteine is present in most proteins and occurs in relative abundance within cells in the tripeptide glutathione (GSH). A large number of different biochemical activities require cysteine thiols, including maintenance of protein structure through disulfide bonds, metal binding, thiol- and thiyl-mediated catalysis, and numerous redox reactions (30, 51, 52). Due to the electronic structure of sulfur, cysteine thiol redox reactions typically involve atom exchange reactions, rather than electron transfer as seen with ROS (51). Such exchange reactions often involve the formation of intramolecular and mixed disulfide bonds. Other cysteine oxidation products include the progressive oxidation products sulfenic (R-SOH), sulfinic ($R\text{-}SO_2H$), and finally sulfonic ($R\text{-}SO_3H$) acid. These latter two thiol oxidation products have been considered irreversible oxidations that could result in permanent loss of protein activity (114, 148). However, a yeast sulfinic acid reductase has been identified and has a human analogue based on sequence homology. Thus, it is possible that sulfinic acid is not an irreversible oxidation (11). Additionally, the RNS •NO and $ONOO^-$ can modify cysteine thiol groups to yield *S*-nitrosothiol and *S*-nitrothiol, respectively (114). Many of these cysteine oxidation products are unstable and transient. For example, sulfenic acid and *S*-nitrosothiol often undergo further oxidation reactions leading to mixed disulfide formation or further oxidation products. Once formed, intramolecular and mixed disulfide linkages are removed by thiol–disulfide exchange reactions and the activities of protein disulfide reductase, glutaredoxin, and thioredoxin reductase (107, 134). The enzymatic specificity and efficiency of enzymatic dethiolating reactions appears to depend on the structural context of the disulfide (110).

Under nonstressed conditions, the cell interior favors a fully reduced thiol state (6, 131). Additionally, although most thiol groups are found on proteins (30), the GSH/glutathione disulfide (GSSG) ratio is considered the major thiol/disulfide redox buffer within cells and normally varies from roughly 30:1 to 100:1 (50, 72, 78). Mixed disulfide levels are usually low in unstressed cells, with ~1% of total protein being *S*-thiolated. Under conditions of oxidant stress, protein-bound thiols can dramatically increase. For example, during the respiratory burst in human neutrophils, up to 17% of cellular GSH can become transiently protein-bound (134). The observation that mixed disulfides readily form during oxidant stress led to the hypothesis that this event is a well-regulated metabolic response as early as 1985 (60). Mixed disulfide formation via GSH addition to protein thiol groups (*S*-glutathiolation) is now considered an important antioxidant defense mechanism, which serves to stabilize oxidized thiol groups, preventing further and possibly irreversible thiol oxidation. Additionally, *S*-glutathiolation has been demonstrated to play an important role in other events, including redox signaling and the modulation of protein activity.

PROTEIN *S*-GLUTATHIOLATION

The cytosolic GSH concentration varies between 1 and 11 m*M* in most cell types, with mitochondrial GSH in the 5–11 m*M* range, making the GSH thiol group concentration much higher than that of most redox-active compounds (24, 50, 59, 87, 91). The importance of GSH in mixed disulfide formation is highlighted by the observation that roughly 85% of mixed protein disulfides contain GSH (22, 123, 133). Other mixed disulfides typically contain cysteine, γ-glutamylcysteine, and trace homocysteine moieties (134). The number of known proteins that can undergo *S*-glutathiolation is relatively large, and a partial list is given in Table 1 (2, 7, 8, 14, 18, 19, 23, 26, 27, 33, 34, 36, 37, 41, 42, 57, 75, 77, 79–84, 92, 95, 97, 102, 111, 112, 117, 126, 132, 139, 141, 144, 145, 147, 148, 150, 159, 165, 166).

It is interesting to note that Klatt *et al*. demonstrated that almost any protein in solution will undergo some *S*-glutathiolation following incubation with GSH and diamide or *S*-nitrosoglutathione (81). Additionally, many reactive protein cysteines may function in an antioxidant/protective capacity only, and their oxidation may have little specificity or effect on protein function (150). Thus, analysis of the significance of *S*-glutathiolation for any protein requires the examination of the *in vivo S*-glutathiolated cysteine pattern, identification and mutation of *S*-glutathiolated cysteine moieties, and examination of any concomitant changes in protein activity accompanying *S*-glutathiolation. Here we will briefly review what is known about a few of the better studied *S*-glutathiolated proteins.

Table 1. A Partial List of Proteins Known to Undergo Protein *S*-Glutathiolation

Protein	*Reference*
Carbonic anhydrase III	14, 77, 81, 150
Protein kinase C	26, 27, 161
Glyceraldehyde-3-phosphate dehydrogenase	42, 81, 84, 139, 148
Alcohol dehydrogenase	81, 139
Enolase	139
Protein tyrosine phosphatase 1B	42, 75
H-ras	2, 77, 95
Glutathione transferases	34
HIV-1 protease	36, 37
c-Jun	79–81
p53	145 (possible)
p50	81
Glycogen phosphorylase	77, 81, 102
Glutaredoxin	81, 147
Thioredoxin	81, 144
Annexin II	144
Superoxide dismutase	81, 132, 148
Calbindin	148
Bovine serum albumin	81, 148
Creatine kinase III	81, 126
Matrix metalloproteinases	112
Caspase-3	81, 166
Calcium ATPase	159
Malate dehydrogenase	41
Aldose reductase	18, 19, 23, 141
Ubiquinating enzymes	75
Hemoglobin	33, 81, 82
Cathepsin K	117
Nuclear factor-1	7
Glycerol phosphate dehydrogenase	81
γ-Glutamyl transpeptidase	83
Aryl sulfotransferase IV	83
Transthyretin	92

Carbonic anhydrase III (CA III)

CA III is one of at least 11 active CA enzymes in humans (90). CA III is unique among the CAs in that it not only is a CA, but also has phosphatase activity when assayed against nitrophenyl phosphate (85). CA III is *S*-glutathiolated specifically at Cys^{181} and Cys^{186} (150). Cabiscol and Levine (14) found that *S*-glutathiolation of Cys^{186} was necessary for phosphatase, but not CA activity. Interestingly, *S*-glutathiolation of Cys^{181} blocked CA III phosphatase activity. The authors hypothesized that the two *S*-glutathiolated cysteines acted as an "on/off" switch that allowed changes in enzyme activity with different cellular oxidative stress levels. Some support for this hypothesis comes from the observation that CA III overexpression protects NIH/3T3 cells from H_2O_2-induced apoptosis (122). Additionally, treatment of rats with acrylonitrile, a reactive and toxic vinyl monomer, resulted in specific binding of radiolabeled acrylonitrile to Cys^{186} of CA III within the rats' livers. As CA III has five total cysteine moieties, this fact further highlights the importance of Cys^{186} in the function and regulation of CA III (108).

Protein kinase C (PKC)

PKC is a family of 10 isozymes that regulate multiple and often opposing functions, including cell-cycle control and proliferation, cell differentiation, and apoptosis (129). PKCδ plays an important role in DNA damage-initiated apoptotic pathways, whereas PKCϵ isozyme activation stimulates cell growth (9, 15, 103). Overexpression of PKCδ in the epidermis of rats resulted in increased resistance to skin tumor promotion by 12-*O*-tetradecanoylphorbol 13-acetate, whereas similar overexpression of PKCϵ resulted in enhanced carcinoma formation after similar tumor promotion (124, 125). Chu *et al.* (26, 27, 161) found that *S*-thiolation (GSH-related peptides) and *S*-glutathiolation strongly inactivated PKCϵ, whereas similar reactions activated PKCδ. For example, PKCδ activity was increased 2–2.5-fold by diamide and $Cys\text{-}Gly_2$, whereas PKCϵ was completely inactivated under the same conditions (27). The authors hypothesized that GSH and other small thiols differentially regulate PKC isozymes under oxidative stress, with PKCδ activation and PKCϵ inactivation having a cumulative antitumor effect (26, 27).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glycolytic enzymes

GAPDH plays a central role in glycolysis and energy production. More recently, GAPDH has been found to have many other functions, including regulation of apoptosis, DNA repair and replication, and nuclear RNA export (139). In yeast, three closely related, but not identical GAPDH isoforms are expressed, Tdh1, Tdh2, and Tdh3, the latter two of which are required for normal cell growth (99, 100). Following treatment of yeast with H_2O_2, Tdh2 and Tdh3 are both *S*-glutathiolated. However, whereas Tdh2 is irreversibly inactivated, Tdh3 activity is restored within 2 h following oxidant removal. Additionally, mutant cells lacking Tdh3 expression are extremely sensitive to the toxic effects of H_2O_2, whereas mutants lacking Tdh2 expression are H_2O_2-resistant (57).

Shenton and Grant (138) extended these observations demonstrating that treatment of yeast with H_2O_2 resulted in an ~70% inhibition of enolase and alcohol dehydrogenase activity under conditions that inhibited Tdh3. All three enzymes also regained significant activity following H_2O_2 removal. The same inhibiting H_2O_2 concentration failed to inhibit aldolase, triose phosphate isomerase, aldehyde dehydrogenase, glucose-6-phosphate dehydrogenase, or 6-phosphogluconate dehydrogenase. The authors hypothesized that glycolytic enzyme inhibition, combined with no inhibition of the enzymes regulating pentose phosphate shunt entry, demonstrated a metabolic-regulatory role for these thiolation reactions. Thus, the flow of glucose equivalents into glycolysis would be shunted away from glycolysis and into the pentose phosphate shunt, increasing the cellular pool of NADPH and the reductive potential and antioxidant capacity of the cell. Less is known about GAPDH *S*-glutathiolation in mammalian cells, although it is known to be *S*-glutathiolated following *S*-nitrosoglutathione decomposition (148). Interestingly, GAPDH has been reported to be extensively *S*-glutathiolated following myocardial ischemia (42, 84).

Protein tyrosine phosphatases (PTPs)

PTPs play an important role in regulating tyrosine phosphorylation and subsequent signaling events, including regulation of cellular differentiation and proliferative responses. The PTP superfamily, although having limited sequence homology, shares a conserved CX5R active-site motif and an identical catalytic mechanism (157). Treatment of cells with growth factors results in transient tyrosine phosphorylation, which involves the generation of intracellular ROS (151). One ubiquitous PTP isoform, PTP 1B, readily undergoes reversible oxidation of Cys^{215} to sulfenic acid following oxidant exposure (39). Barrett *et al.* (8) found that treatment of PTP 1B with diamide and GSH resulted in specific Cys^{215} *S*-glutathiolation and phosphatase inactivation. Additionally, treatment with dithiothreitol or glutaredoxin resulted in phosphatase reactivation, indicating Cys^{215} *S*-glutathiolation both protected Cys^{215} from further oxidation and concomitantly regulated phosphatase activity. Interestingly, van Montfort *et al.* (158) found that oxidation of Cys^{215} resulted in the formation of a sulfenyl-amide species that likely functions to both inhibit irreversible oxidations of Cys^{215} and facilitate its thiol-mediated reactivation.

H-ras

H-ras is a member of a GTPase superfamily known to be involved in a large number of cellular functions, including cell survival, growth, cytokine production, and differentiation (43). Considerable evidence indicates that H-ras is important in oxidant-induced signal transduction and, in fact, when active produces relatively large amounts of $O_2^{-\bullet}$, which appears to play an important role in cell-cycle progression (1, 32, 61, 73, 105, 143, 146). H-ras has six cysteine moieties, four of which are surface-exposed (65, 89, 115). Mallis *et al.* (95) found that H-ras could undergo complex cysteine modifications by multiple oxidants. For example, *S*-nitrosoglutathione *S*-nitrosylated H-ras Cys^{118}, Cys^{181}, Cys^{184}, and Cys^{186}, whereas diamide treatment resulted in at least two *S*-glutathiolation events. The authors hypothesized that different levels of oxidative/nitrosative stress result in multiple H-ras cysteine thiol group modifications, resulting in complex protein regulatory activity. Support for this hypothesis comes from the recent finding that H-ras Cys^{181} *S*-glutathiolation in vascular smooth muscle cells following angiotensin II treatment results in increased H-ras activity. The increase in activity was antagonized by cotreatment with dithiothreitol or glutaredoxin overexpression, indicating that Cys^{181} *S*-glutathiolation is an activating event (2). Additionally, several H-ras reactive cysteine moieties are modified by events such as palmitoylation and farnesylation, which regulate membrane localization (20, 120). Thus, *S*-nitrosylation and *S*-glutathiolation may regulate H-ras subcellular localization, as well as activation.

Microsomal glutathione S*-transferase (mGST)*

The GSTs include nearly 20 cytosolic forms and one microsomal form. The GSTs have multiple known functions, including detoxification, particularly of exogenous toxicants, peroxidase activity, and protection from oxidative damage (128). Dafre *et al.* (34) found that incubating mGST with GSSG increased enzymatic activity roughly fivefold, and that this event is reversed by glutathione reductase exposure. Additionally, treatment of microsomes with GSH and *tert*-butyl hydroperoxide or cumene hydroperoxide resulted in an increase in the mGST molecular weight of an amount approximately equivalent to the addition of a GSH moiety. This higher molecular mGST was not seen in the presence of the reductant mercaptoethanol. The authors suggested that under oxidant stress, mGST is activated via *S*-glutathiolation, leading to oxidative stress resistance. GST regulation by *S*-glutathiolation appears to be somewhat limited, as other researchers have found no effect between the different GST forms generated by *S*-glutathiolation events (21, 93).

HIV-1 protease

Human immunodeficiency virus (HIV) encodes an aspartyl protease required for viral maturation. The protease contains two cysteines that are highly conserved among different viral isolates and are not required for catalysis (35). Davis *et al.* (36, 37) examined these cysteine moieties and found that Cys^{95} *S*-glutathiolation abolished protease activity, whereas *S*-glutathiolation of Cys^{67} both stabilized the protease and increased the k_{cat} roughly twofold, without effecting the K_m. Additionally, diglutathiolated forms of the HIV-1 protease exhibited differential susceptibility to dethiolation by thioltransferase. The Cys^{95}-SSG was most effectively dethiolated by thioltransferase, whereas Cys^{67}-SSG exhibited comparative resistance to enzymatic dethiolation. Thioltransferase treatment of the diglutathionylated protease also restored protease activity and increased protease specific activity three- to fivefold compared with the fully reduced form. The authors concluded that cysteine modification, especially *S*-glutathiolation, combined with thioltransferase activity plays an important role regulating protease activity.

CYSTEINE THIOL MODIFICATION AND TRANSCRIPTION FACTOR ACTIVITY

The activity of several transcription factors is known to be modulated by cysteine moiety modification. Among these transcription factors are activating protein-1 (AP-1), p53, nuclear factor-κB (NF-κB), cyclic AMP-response element binding protein (CREB), and SP-1 (12, 38, 56, 62, 71, 79–81, 101, 109, 121, 137, 145, 162–164). Here we will briefly review c-Jun and p53 and *S*-glutathiolation.

c-Jun

c-Jun is a major component of the AP-1 transcription factor, which regulates a large number of genes involved in cell proliferation, differentiation, and apoptosis. Specifically, c-Jun plays a major role in cell proliferation, and its dysregulation is a common event in carcinogenesis (137). Klatt *et al.* (79, 80) found that exposure to GSSG or *S*-nitrosoglutathione resulted in *S*-glutathiolation of a specific cysteine moiety in the c-Jun DNA-binding region. This *S*-glutathiolation re-

sulted in binding inhibition that was reversed by GSH exposure. Binding-site analysis demonstrated that *S*-glutathiolation sterically blocked DNA binding. The authors concluded that transcription factor *S*-glutathiolation represented an important mechanism by which oxidative stress could immediately alter gene transcription patterns. Support for this hypothesis comes from the observation that induction of AP-1-dependent apoptosis in Jurkat cells was inhibited by •NO donors. •NO acted via reducing the number of titratable cysteine c-Jun thiol groups, which in turn correlated with lowered c-Jun DNA binding, lowered AP-1-driven promoter activity, and attenuated caspase activation (101). Although the chemical nature of the •NO-induced thiol modification was not addressed, the study demonstrated that oxidant-induced modifications of c-Jun cysteine moieties can significantly alter both c-Jun DNA binding and c-Jun-mediated gene expression and signal transduction pathways.

p53

p53 is mutated in roughly 30–50% of human cancers, and germ line mutation of p53 results in Li–Fraumeni syndrome, a hereditary cancer predisposition resulting in increased incidence of sarcomas, lymphomas, breast, brain, and other tumors (94, 113, 141). Mice deficient in p53 expression develop normally, but are highly susceptible to cancer, whereas mice that overexpress p53 exhibit shortened life span and an age-associated phenotype, including osteoporosis and organ atrophy (40, 155). p53 normally functions as a tetramer and plays a vital role in genomic integrity maintenance, cell-cycle control and checkpoint responses, DNA repair, differentiation, and apoptosis (for review, see 70). p53 has 10 cysteine moieties, all of which are located within the DNA-binding region of p53 (58). Numerous studies indicate that these cysteine moieties are required for p53 activity. For example, Cys^{176}, Cys^{238}, and Cys^{242} are required to coordinate binding with a zinc atom, and mutation of any of these cysteine residues ablates DNA binding (121).

Multiple studies demonstrate that cysteine oxidation inhibits p53 DNA binding, whereas antioxidants such as dithiothreitol often increase p53 DNA binding (12, 38, 62, 71, 79, 121, 162, 163). Cysteine moiety oxidation has very specific effects in regulating p53 activity. For example, Buzek *et al.* (12) found that different redox states of Cys^{277} allowed p53 to discriminate between different p53-responsive DNA-binding elements. Presently, p53 is not known to be *S*-glutathiolated. However, Sun *et al.* (145) performed a detailed analysis of p53 cysteine oxidation and found that cysteine-thiol-oxidized p53 failed to bind the GADD45 response element, and existed mainly as a monomer. Reduced p53 existed predominantly as a tetramer and readily bound the GADD45 response element. Based on protein modeling studies, Sun *et al.* suggested that a Cys^{182} gluthathione disulfide may form following oxidative stress, resulting in tetramer dissociation, thus accounting for many of the activities exhibited by oxidized p53.

Other transcription factors

The activities of several other transcription factors have been found to be modulated by cysteine thiol modifications, including NF-κB, nuclear factor-1 (NF1), CREB, and SP-1 (56, 101, 109, 137, 164). As yet, CREB, and SP-1 have not been identified as undergoing *S*-glutathiolation, whereas NF1 and the p50 subunit of NF-κB can be *S*-glutathiolated *in vitro* (7, 81). NF-κB DNA binding can be regulated by cysteine moiety oxidation/modification (109). NF1 *S*-glutathiolation inhibits DNA binding and is readily reversed by *N*-acetylcysteine *in vivo* and glutaredoxin coupled to GSH and GSSG reductase in *in vitro* binding assays (7).

CONCLUSION

S-Glutathiolation is now known to be a common cellular response following oxidative stress. The above examples demonstrate that *S*-glutathiolation has multiple functions, including the following: (a) protection of vulnerable protein thiol groups from irreversible oxidation reactions; (b) regulation of specific protein activity following oxidative stress (seen with CA III); (c) coordination of cellular metabolism following oxidative stress (seen in the modulation of glycolysis and the pentose phosphate shunt in yeast); (d) coordination of gene expression patterns via modulation of transcription factor activity (seen with p53); (e) regulation of protein processing and ubiquitination; (f) the modulation of cell division (PTP 1B); (g) inhibition of the tumor-promoting effects of oxidants (seen in the differential modulation of PKCϵ and PKCδ); and (h) integration of ROS/RNS-initiated signal transduction cascades. Thus, protein *S*-glutathiolation represents an important, versatile, and vital cellular response to oxidative stress. Additionally, protein *S*-glutathiolation increases with toxic exposure and in certain diseases, such as sickle cell anemia, diabetes mellitus, familial transthyretin amyloidosis, and possibly Parkinson's disease (3, 55, 92, 121). Protein *S*-glutathiolation may also increase with aging (96, 149). Thus, protein *S*-glutathiolation, like ROS/RNS activities, may play a vital role in both normal and pathophysiological processes.

S-Glutathiolation is one of multiple thiol modifications that regulate cell and protein activity. For example, over 100 different proteins are known to be *S*-nitrosylated, a modification that in some cases regulates protein activity in a manner analogous to protein *S*-glutathiolation (49, 74, 98). Inter- and intramolecular disulfide formation is also a major regulator of protein function, whereas the intracellular GSH pool regulates cell growth, apoptosis, and differentiation, and other functions such as exogenous compound detoxification (6, 25, 29, 114, 116, 145). Thus, thiol group protein modifications and thiol moiety redox activity are becoming increasingly important in the study of normal cellular function and oxidative stress and subsequent adaptive and pathological cellular responses.

ABBREVIATIONS

AP-1, activating protein-1; CA, carbonic anhydrase; CREB, cyclic AMP-response element binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH,

glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; HIV, human immunodeficiency virus; H_2O_2, hydrogen peroxide; mGST, microsomal glutathione *S*-transferase; NF1, nuclear factor-1; NF-κB, nuclear factor-κB; $^{\bullet}NO$, nitric oxide; $O_2^{-\bullet}$, superoxide; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite anion; PKC, protein kinase C; PTP, protein tyrosine phosphatase; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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