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Biochemical Pharmacology 64 (2002) 1019–1026

Biochemical Pharmacology

Cellular glutathione and thiols metabolism

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Received 31 January 2002; accepted 6 March 2002

Abstract

Low molecular weight thiol-containing compounds have an essential role in many biochemical and pharmacological reactions due to the ease with each they are oxidized, and the rapidity with which they can be regenerated. Thioredoxin and glutathione (GSH) are two of the major small molecular weight thiol-containing compounds synthesized *de novo* in mammalian cells that participate in those functions. Understanding the mechanisms of thiol metabolism has special relevance to understanding the cell's defense against toxicant exposure and as the focal point in redox signaling. This commentary will, however, focus on GSH consumption and synthesis, and the role of thiols in signaling. The chemical reactions of GSH, including conjugation reactions mediated by glutathione S-transferases (GST) and oxidation reactions mediated by glutathione peroxidases will be described. The regulation of GSH synthesis will be illustrated from a compilation of studies designed to understand the various levels at which enzymatic GSH biosynthesis is controlled, and the signaling pathways that mediate them. The response of the cell to 4-hydroxynonenal (4HNE), a reactive aldehyde produced physiologically in response to inflammation and various air pollutants, will be explored in detail. Finally, the direct role of thiols as signaling molecules will be addressed, with particular attention given to ''redox state.'' It is our aim that this commentary will lead the reader to appreciate that studies investigating the signaling for and regulation of thiol metabolism must never be generalized, and that perturbations in any of step of thiol metabolism may have etiological roles in genetically, virally, and environmentally borne pathologies.

Keywords: Signal transduction; Glutamate cysteine ligase; Glutathione synthetase; Thioredoxin; Hydrogen peroxide; 4-Hydroxynonenal

1. Overview

Thiol-containing compounds are central in many biochemical and pharmacological reactions. Disulfide bonds have an important role in determining the tertiary structure of proteins, and in many drugs the cysteine moiety is an important reactive center that determines their effects. Molecules containing cysteine residues are among the most easily metabolized compounds, being easily oxidized by transition metals or participating in thiol–disulfide exchange. Nevertheless, with exceptions that are described in detail below, cysteines do not react with hydroperoxides directly at physiological pH. Glutathione (GSH), which has one cysteine, and the small protein thioredoxin, which has two cysteines in its active site, often have complementary, if not overlapping roles in cytoprotection. GSH is the most abundant non-protein thiol, being found in the millimolar range in most cells. Thioredoxin is the smallest of a family of proteins containing a ''thioredoxin fold,'' which play important roles in antioxidant defense, protein folding and signal transduction.

The response of a cell to a stress often involves changes in thiol content, which is first consumed in reactions that protect the cell by removing the deleterious compound, and is then replaced through either enzymatic reduction of a disulfide, when that is possible, or by *de novo* synthesis. Importantly, these changes in thiol content and metabolism can have effects on signaling pathways.

2. Roles of GSH as a protective agent

As the predominant non-protein sulfhydryl in cells, GSH plays several important roles. It has long been established

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Abbreviations: GSH, glutathione; GSSG, glutathione disulfide; GSHPx, glutathione peroxidase; GST, glutathione S-transferases; GGT, γ-glutamyl transpeptidase; GCL, glutamate-cysteine ligase; GCLM, glutamate-cysteine ligase modulatory subunit; GCLC, glutamate-cysteine ligase catalytic subunit; GS, glutathione synthase; TRE, activator protein-1 responsive element; 4HNE, 4-hydroxynonenal; PTP, protein tyrosine phosphatases; Trx, thioredoxin.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01172-3

that the thiol moiety of GSH is important in antioxidant defense, xenobiotic and eicosanoid metabolism, and regulation of the cell cycle and gene expression (for reviews, see [1–5]). Although GSH does not react directly with hydroperoxides, its use as a substrate for glutathione peroxidase (GSHPx) has been recognized for almost 40 years as the predominant mechanism for reduction of H_2O_2 and lipid hydroperoxides [6]:

$$H_2O_2 + 2GSH \xrightarrow{\text{Glutathione peroxidase}} 2H_2O + GSSG \quad (1)$$

$$ROOH + 2GSH \xrightarrow{\text{Glutathione peroxidase}} 2ROH + GSSG \quad (2)$$

where ROOH and ROH are a hydroperoxide and its corresponding alcohol. The GSHPxs are a family of selenoproteins that vary in their hydroperoxide substrate specificity [7]. More recently, a family of proteins, now called peroxiredoxins, has been recognized as catalyzing the reduction of H_2O_2 by GSH and/or other thiols, but with cysteine, in its thiolate (S^-) form in their active sites rather than selenium. The reaction sequence is:

$$H_2O_2 + Prx\text{-}S^- \rightarrow OH^- + Prx\text{-}SOH \quad (3)$$

$$Prx\text{-}SOH + GSH \rightarrow Prx\text{-}SSG + H_2O \quad (4)$$

$$Prx\text{-}SSG + GSH \rightarrow Prx\text{-}S^- + GSSG \quad (5)$$

where Prx-SOH represents the sulfenic acid intermediate.

Glutathione disulfide (GSSG), which should not be referred to as oxidized glutathione, is reduced to GSH by NADPH through the glutathione reductase reaction:

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} NADP^+ + 2GSH \quad (6)$$

while NADPH is maintained predominantly by the pentose phosphate shunt. GSSG is normally maintained as less than 1% of total glutathione. Increases in GSSG during oxidative stress are generally transient as reduction by glutathione reductase is relatively rapid. Nonetheless, GSSG can exchange with protein sulfhydryls to produce protein–glutathione mixed disulfides [8]:

$$GSSG + protein{-}SH \rightleftharpoons protein{-}SSG + GSH \quad (7)$$

The mixed disulfides (protein–SSG) have a longer half-life than GSSG, probably due to protein folding, and a significant basal level is found in cells [9]. This exchange reaction provides an important mechanism for the action of GSH in cell signaling (see below). An ATP-dependent transport mechanism is also responsible for decreasing GSSG through export [10]. Although these aspects of GSH thiol chemistry are generally well established, the literature abounds with cases in which the details are disregarded in the interpretation of experimental results.

GSH forms conjugates with a great variety of electrophilic compounds nonenzymatically, when the electrophile is very reactive, or more often through the action of glutathione S-transferases (GST). Conjugation with GSH is an essential aspect of both xenobiotic and normal physiological metabolism (for reviews, see [11,12]). Formation of conjugates can result in depletion of GSH and has been used as a tool to study the role of GSH in antioxidant defense. A caveat is that the use of a strong electrophile that does not require catalysis will react with protein thiols as well, producing non-specific responses.

Glutathione conjugates with small molecules are excreted from cells [13]. While this is generally considered as part of detoxification, glutathione-conjugates of compounds, such as hexachlorobutadiene, can produce renal toxicity [14]. While most other tissues have low γ-glutamyl transpeptidase (GGT) activity, the high GGT activity in kidneys, which breaks down glutathione-conjugates to mercapturic acids, allows their uptake by proximal tubular cells. Nonetheless, aside from the kidney, the activities of GGT and glutathione-conjugate excretion are considered an important component of defense against xenobiotics.

While GSH does not react nonenzymatically with H_2O_2, another role for GSH in antioxidant defense that depends upon its ability to react with carbon centered radicals ($R^\bullet$) has been proposed by Winterbourn [15]. In this "free radical sink" hypothesis, GSH acts in concert with superoxide dismutase to prevent oxidative damage:

$$R^\bullet + GSH \rightarrow RH + GS^\bullet \quad (8)$$

$$GS^\bullet + GS^- \rightarrow GSSG^{\bullet -} \quad (9)$$

$$GSSG^{\bullet -} + O_2 \rightarrow GSSG + O_2^{\bullet -} \quad (10)$$

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2 \quad (11)$$

The critical role of GSH in protecting cells should be apparent from the multitude of reactions through which it removes potentially harmful molecules from cells. While restoration of GSH from GSSG can be easily accomplished, depletion through conjugation or loss by excretion of GSSG demands replenishment. Although a few cells can take up GSH, *de novo* synthesis is the predominant pathway for this restoration.

3. The enzymatic synthesis of glutathione

The synthesis of GSH from its constituent amino acids is both constitutive and regulated, and results from the concerted effort of two ATP-dependent enzymes:

$$\text{L-glutamate} + \text{L-cysteine} + ATP \xrightarrow{GCL} \gamma\text{-L-glutamyl-L-cysteine} + ADP + P_i \quad (12)$$

$$\gamma\text{-L-glutamyl-L-cysteine} + \text{glycine} + ATP \xrightarrow{GS} \gamma\text{-L-glutamyl-L-cysteinyl-glycine} + ADP + P_i \quad (13)$$

This first enzyme, following IUBMB recommendations is named glutamate-cysteine ligase (GCL, E.C. 6.3.2.2), formerly called γ-glutamylcysteine synthetase (GCS), and is rate-limiting. GCL is a heterodimer which can be

dissociated under non-denaturing conditions into a modulatory, or light, subunit (GCLM), and a catalytic, or heavy, subunit (GCLC) [16]. GCLC is 73 kDa in size, possesses all of the catalytic activity of GCL, and is the site of GSH feedback inhibition. The lighter 31 kDa GCLM subunit, which as a monomer has no known catalytic function, exhibits a modulatory or regulatory effect, on the GCLC subunit when associated. This association is probably essential for GSH biosynthesis under normal physiological concentrations of glutamate and GSH. Studies performed *in vitro* using purified rat [17] or recombinant human enzymes [18,19], and observations made *in vivo* from transgenic mice [20] suggest that the major effect of the light subunit *in vivo* is elevating the K_i for GSH such that it decreases negative feedback inhibition. The activity of the GCL holoenzyme can further be regulated either positively or negatively by S-nitrosation [19], phosphorylation [21] and oxidation [22], although increased GCL activity in most cases involves a transcriptional component leading to increased production. The second enzyme required for *de novo* GSH biosynthesis is glutathione synthase (GS, E.C. 6.3.2.3), sometimes called glutathione synthetase. This enzyme functions as a homodimer of 118 kDa, and is responsible for the addition of glycine to γ-glutamylcysteine created by GCL to form GSH, γ-L-glutamyl-L-cysteinyl-glycine. While less is known about the regulation of GS activity compared with GCL, certain clinical phenotypes have been found to result from the inheritance of missense mutations in *Gs*. Most of these mutations decreased either the K_m for glycine and/or the V_{max} value, or caused a decrease in the stability of the enzyme [23].

4. Regulation of the glutathione biosynthetic genes

Each of the three key enzymes responsible for GSH biosynthesis are encoded by a single-copy gene in the haploid human genome. The cDNAs for both *Gcl* genes have been cloned and sequenced [24,25], and the 5′ untranslated regions of both have been cloned, sequenced and analyzed for regulatory elements that could mediate transcription in response to a stimulus [26–28]. The cDNA for *Gs* has been cloned and sequenced [29]. Unfortunately, as the 5′ untranslated region of *Gs* has not been cloned, and is not well represented in the expressed sequence tag databases, potential regulatory elements for this gene remain unknown.

A remarkably large number of compounds, including those forming glutathione-conjugates and those generating reactive oxygen species, have been shown to induce glutathione biosynthesis through increased transcription of GCL (see [30] for review). Sequence analysis and experimental manipulations employing reporter constructs of the 5′ untranslated regions for human *Gclc* and *Gclm* genes have revealed several putative enhancer elements that could mediate, either alone or in combination, an increase in transcription in response to the binding of transcription factors, whose activity has been increased in response to a stimulus signaled by the presence of some compound. The *Gclc* promoter contains many potential *cis*-acting elements, including consensus recognition sites for binding of Sp-1, activator protein-1 (TRE), TRE-like, activator protein-2 (AP-2), nuclear factor kappa B (κB), and the electrophile response element (EpRE, sometimes called the antioxidant response element, ARE) binding complexes [26,27]. The *Gclm* promoter contains many of the same elements present in the *Gclc* promoter, with the notable exception of the κB element [28]. Of the above-mentioned enhancer elements, those that have received the most attention have been TRE or TRE-like elements, and EpRE elements. The role of these elements in mediating *Gcl* transcription in response to various stimuli has been reviewed previously [31]. In summary, it appears that both EpRE and TRE sites are involved in *Gcl* induction and that differences in the expression of signaling components and metabolism among cell types cause signaling for activation of the corresponding transcription factors to vary even with the same compound.

5. Glutathione content is responsive to environmental factors

Many different conditions are known to change intracellular GSH content. These include the presence of heavy metals [32], high glucose concentrations [33] and heat shock [34]. Exposure to reactive oxygen and nitrogen species including H_2O_2 [35] and nitric oxide [36], or to compounds that can generate reactive species including 2,3-dimethoxy-1,4-naphthoquinone [37], menadione [35,37], *tert*-butylhydroquinone [38,39], pyrrolidine dithiocarbamate [40] and β-naphthoflavone [27], and other reactive biological products such as 15-deoxy-Δ(12,14)-prostaglandin J2 [41], oxidized low density lipoproteins [42], and 4-hydroxy-2-nonenal [43–45] can increase the content of GSH by increasing the rate of GSH synthesis.

The intracellular content of GSH is a function of the balance between use and synthesis. Depletion of GSH occurs by conjugation reactions via the GST, or by GSSG formation through increased H_2O_2 production and GSHPx activity. The actions of these enzymes are beneficial to the cell by removing deleterious species, but do so at the expense of GSH, which must be replaced. The GSSG formed by peroxidases can be reduced to regenerate GSH by glutathione reductase (GR, E.C. 1.6.4.2) at the expense of NADPH, although the replacement of GSH is due principally to *de novo* synthesis.

Because GCL is feedback inhibited by GSH, depletion of GSH can result in a short-term increase in GSH synthesis. To some extent, a decrease in GSH will cause a transient increase in the activity of pre-existing GCL by

decreasing the feedback inhibition by GSH, resulting in a short-term increase in GSH synthesis [46]. Nonetheless, increased *de novo* synthesis is due primarily to increased synthesis of GCL subunits through a combination of increased transcription and mRNA stability [43,47,48]. The mechanisms by which compounds change GSH content have been investigated in many cases, especially at the level of *Gcl* gene induction. A recent detailed report shows clearly that these genes are differentially regulated [49]. Perhaps the most important insight gained from reviewing these studies is the differences among inducers upon *Gcl* gene induction, GCL subunit content, and when studied, the differences in signaling pathways. For a thorough review of the complexities of the control of *Gcl* transcription, see [30]. One particularly well-studied inducer of GSH biosynthesis, and an intriguing example, is the lipid peroxidation end-product 4-hydroxynonenal (4HNE).

6. 4HNE and glutathione metabolism

4HNE is an α,β-unsaturated aldehyde that is formed from the reaction of reactive oxygen species with the *n*-6 polyunsaturated lipids in cellular membranes during inflammation and exposure to air pollutants, such as nitrogen dioxide and ozone [50–52], imparting 4HNE particular relevance to public health. Furthermore, 4HNE is relatively stable *in vivo*, and as such has been proposed as being one of the key mediators of the damage resulting from exposure to reactive oxygen and nitrogen species [53]. 4HNE is removed from many cell types by reactions with GSH, catalyzed by the GST subclasses that have relative specificity for alkenals (GSTA4-4 and GST5.8), the expression of which are regulated by their substrates, including 4HNE [54,55]. Another major pathway for removal of 4HNE is its conversion to 4-hydroxynonenol by an aldehyde reductase [56] that is also inducible by 4HNE [57], while a third pathway is its oxidation by an aldehyde dehydrogenase to 4-hydroxy-2-nonenoic acid [58]. The relative contributions of each of these pathways in the removal of 4HNE has been reported for aortic endothelial cells [59] and isolated perfused rat heart [60]. Exposure of a rat alveolar epithelial cell line (L2 cells) to 4HNE caused an increase in GSH biosynthesis [43]. Similar results were obtained with a normal human bronchial epithelial cell line, HBE1 (unpublished data).

4HNE has been demonstrated to activate the JNK pathway [61–63], which in turn activates the activator protein-1 (AP-1) transcription factor complex through phosphorylation of Jun family members, c-Jun and JunB. AP-1 is formed by dimerization of Jun family proteins as either homodimers or heterodimers with other Jun family members, Fos family members, or other proteins, such as ATF-2. These various AP-1 dimers bind to TRE elements. Jun family members can also pair with Nrf2 and small Maf proteins to form EpRE-binding complexes [64].

Recently, we demonstrated that exposure of HBE1 cells to physiologically relevant levels of 4HNE caused a dose- and time-dependent increase in the intracellular content of GSH. This was closely correlated with an increase in the content of both GCL subunits. Not surprisingly, this increase in the subunit content was correlated with, and kinetically preceded by, an increase in the steady-state level of both *Gcl* mRNA species, also occurring in both dose- and time-dependent fashions. Based on the putative *cis*-acting elements in the *Gcl* promoter identified as being likely to mediate transcription, using the electrophoretic mobility shift assay, we investigated the activation of relevant transcription factor complexes in response to 4HNE exposure, and showed that only the AP-1 binding complex was activated, whereas EpRE and NF−κB binding complexes showed no change in activity. Super-shift analyses demonstrated the presence of various members of the Jun family. Furthermore, we showed that the content of phosphorylated c-jun, a common component of activated AP-1 transcription factor complexes, increased with 4HNE exposure. These collective results suggested that 4HNE was signaling for increased GSH biosynthesis via the JNK pathway.

Results from previous studies have suggested roles for TRE and EpRE elements in mediating transcription, with signaling being mediated by MAPK pathways. The role of MAPK pathways in mediating the *Gcl* response to 4HNE was examined. Inhibition of the p38 pathway with the pharmacological inhibitor SB202190 had no effect on AP-1 binding activity, or on the steady-state level of *Gcl* mRNAs. Similarly, inhibition of the ERK signaling pathway with the pharmacological inhibitor PD98059 had no effect on AP-1 binding activity, or on steady-state *Gcl* mRNA content. Using deductive reasoning, these results suggest that either the JNK pathway mediates the effects of 4HNE, or that non-MAPK pathways are responsible. This question can finally be addressed by the use of either peptide-based, membrane-permeable inhibitors of JNK signaling [65] or with the use of the new pharmacological inhibitor, SP600125 [66]. Results from these experiments will reveal the role of MAPK signaling, and JNK signaling specifically, in mediating GSH synthesis in response to 4HNE. These results are eagerly anticipated.

Similar work done by us, previously using 4HNE in rat L2 cells, showed no effect of inhibiting the p38 pathway, while inhibition of the ERK pathway showed an effect on the steady-state mRNA content of *Gclc* but not for *Gclm*. This suggested to us partial involvement of the JNK pathway in *Gclc* expression, with probable significant involvement for *Gclm* [44]. Moreover, an interesting difference in signaling for the two *Gcl* genes in L2 cells by 4HNE was demonstrated previous to this, with a requirement for *de novo* protein synthesis for *Gclm* transcription but not for *Gclc* transcription, further revealing that the signaling pathways are indeed different [43]. These three studies, conducted by the same group with the same inducer in

similar cells types, nicely underscores the species-specific differences that exist between rat and human pathways controlling for GSH biosynthesis. We believe the results from these studies diminish the relevance of further use of rat models for studying aspects of GCL regulation. Not surprisingly, other research illustrates the importance of not generalizing results obtained from one inducer to another. Using human hepatoma cells, Mulcahy and coworker have demonstrated equivalent roles of both the p38 and ERK pathways in mediating the signaling for GSH production and *Gcl* transcription in response to PDTC [67].

7. Roles of GSH in signaling

As of the date this is being written, PubMed lists 2276 papers in a search for ''redox state.'' Recently, Buettner and coworker [68] examined the question of how to define the cellular redox state and determined that the ratio (2GSH:GSSG) is the best definition of that term. The next question then, is how does that ratio translate into the chemistry of redox signaling? A seemingly obvious hypothesis is disulfide exchange between GSSG and protein thiols. But, as protein thiols are ubiquitous, the specificity required for signaling is absent in this reaction. In the interior of the endoplasmic reticulum, where the (2GSH:GSSG) is low, mixed disulfide formation and disulfide exchange is an important component of protein folding. Certainly, when there is a sufficient oxidative stress to cause a significant increase in GSSG content through the GSHPx reaction (reaction (1)) above the normal less than 1% of total glutathione, protein mixed disulfides will increase. A significant number of proteins involved in signaling that have critical thiols, such as receptors, proteins involved in ubiquitinylation, several protein kinases, and some transcription factors, can be altered in their function by formation of mixed disulfides. While such changes represent a broad response to oxidative stress that is influenced by glutathione metabolism rather than specific signaling, they clearly represent an important aspect of pathophysiology and probably trigger adaptive responses when the stress is not lethal. In this regard, GSSG appears to act as a fairly non-specific signaling molecule. Under normal physiological conditions, however, GSH may function in signaling in a less direct fashion; in being a determinant of the rate of H_2O_2 removal by GSHPx as H_2O_2 is the more likely direct agent of redox signaling.

The necessary specificity for H_2O_2 reactions in signaling apparently involves a different aspect of thiol chemistry than its reaction in the GSHPx reaction or nonenzymatic metal catalyzed oxidations. In the absence of catalysis, H_2O_2 is unreactive with thiols. While metal-catalyzed reactions of H_2O_2 or peroxynitrite with a thiol can produce either a sulfinic ($-SO_2H$) or sulfonic ($-SO_3H$) acid, which are not easily reduced, low concentrations of H_2O_2 react spontaneously with the thiolate anion (S^-) to form a sulfenic acid as in reaction (3). Thiolates are not common in the cell as cysteine normally has a pK_a significantly higher than the physiological range. Despite that, thiolates are found in the active sites of a group of proteins that include peroxiredoxins, protein tyrosine phosphatases (PTP) and the thioredoxin (Trx) family of proteins. The active sites of these proteins provide an unusually basic microenvironment in which cysteine dissociates to form the thiolate. Furthermore, as these thiolates are critical to the function of these proteins, the reaction with H_2O_2 provides a mechanism for direct involvement of H_2O_2 in signaling when the target molecule, such as Trx or a PTP, is part of a signaling pathway [69–72].

Alteration of the PTP thiolate results in loss of activity and this has been suggested by *in vitro* and indirect cellular experiments as a mechanism for H_2O_2 signaling [70,73]. This is one place where GSH could have a direct role in signaling. As described for the peroxiredoxin reaction, the sulfenic acid can be easily reduced in two sequential reactions involving GSH (as in reactions (4) and (5)), restoring the PTP activity. Reversibility is an important component of signaling as it allows the ''turn-off'' of a signaling pathway.

Although the catalytic site of Trx family proteins also has a thiolate that can form a sulfenic acid, a second conserved cysteine reacts with the sulfenic acid to form an intramolecular disulfide bridge. Such an intramolecular disulfide bond cannot be reduced by GSH, but is reduced by NADPH catalyzed by Trx reductase. Trx has been implicated in H_2O_2 signaling through sulfenic acid formation. Indeed, activation of ASK1, an upstream kinase in the JNK pathway, seems to be clearly regulated through such a scenario of reversible Trx oxidation [74]. The role of thiols as the focal point of redox signal transduction is likely to be the next major area of intense investigation in this field [75].

8. Some final thoughts

GSH is a major component of the process for defense against the toxicity of xenobiotic compounds and oxidants to which exposure is an everyday occurrence. Normal metabolism requires constant and rapid replenishment of GSH, which is accomplished through both the reduction of GSSG and *de novo* synthesis. Chronic exposure appears to require an even greater capacity for GSH synthesis. Compelling new evidence suggests that alterations in GSH metabolism may also be an underlying mechanism in HIV infection, which for some time has been known to be associated with a systemic decrease in the GSH content [76,77], although significant controversy remains concerning the mechanisms of GSH depletion. Recently, our lab has demonstrated in the livers and erythrocytes of Tat^+ transgenic mice this decrease in GSH results from a

decrease in *Gclm* mRNA and protein content, and a significant reduction in the activity of GS [20]. Specifically, we showed that downregulation of *Gclm* in Tat^{+} mice was associated with an increased sensitivity of GCL to feedback inhibition by GSH, which is likely to be partially responsible for the observed decreased level of GSH. Furthermore, GS activity was also decreased, and was found to linearly correlate with the GSH content. We propose the HIV Tat protein causes a perturbation in the intracellular GSH level, leaving the cells more vulnerable to damage by oxidants and xenobiotics. Increased drug toxicity and oxidative damage is often found in HIV-infected individuals and suggests the importance of GSH in disease progression; GSH content may even predict the survival of HIV-seropositive individuals [78,79].

Determining which signaling pathways lead to alterations in thiol metabolism is critical for understanding the mechanisms of, and developing therapies for, environmental toxicants. We hypothesize that many environmental agents exert their deleterious effects by altering, either directly or indirectly, the cellular redox status through manipulation of the metabolism of thiols such as glutathione. This was exemplified in the above examples with 4HNE, a reactive aldehyde produced in normal metabolism but markedly elevated during inflammation or in response to exposure to pollutants such as NO_2. Knowing how such agents alter these essential and ubiquitous biochemical pathways should facilitate the understanding of redox-mediated changes in other pathways and pathologies.

Acknowledgments

This work was supported by grant ES05511 from the National Institutes of Health. We thank the workers in our lab that contributed to our work, particularly Rui-Ming Liu and Karen Iles, and to the many people who have contributed to understanding the roles of thiols in biology, most of whose work could not be cited in such a brief commentary.

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