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Glutathione - from antioxidant to post-translational modifier

Henry Jay Forman¹

¹Ethel Percy Andrus Gerontology Center, Leonard Davis School of Gerontology, The University of Southern California

Abstract

Helmut Sies is one of the leading investigators in the multiple roles of glutathione (GSH) in biology. He has pioneered work on the role of GSH in preventing oxidative stress, in transport of GSSG, in protection of protein thiols from irreversible oxidation through mixed disulfide formation and demonstrated a role of protein glutathionylation in response to hormonal stimulation well before redox signaling became a major subject of investigation. Here I will describe the roles of GSH in several aspects of biology, the work of my laboratory in those findings, and how Helmut Sies work influenced our studies.

Keywords

Glutathione; protein mixed disulfide; γ -glutamylcysteine ligase; scavenger pathway; redox signaling

A very brief history of the discovery of glutathione

Helmut Sies has played a major role in our understanding of the role of glutathione (GSH) in biochemistry and physiology (173 references found in PubMed for “Sies H and glutathione”). So, the topic of GSH is central to this special issue. Research on GSH was my original connection to Helmut. It came in the form of a review of a manuscript (not likely to have been by Helmut) that pointed out that we had made a complete mess of the measurement of GSSG, the disulfide form of GSH and that the correct way of doing it was in the *Methods in Enzymology* article by Akerboom and Sies (1) or if we had an HPLC, the method of Farris and Reed (2). We had used a published method (using 2-vinylpyridine to modify GSH without removal of the excess) that was inappropriate for cells, but was “convenient” for an Assistant Professor without an HPLC. So, we used the Akerboom and Sies method in which N-ethylmaleimide was used to modify GSH and then excess removed by chromatography (1) and our revised paper was published (3). We determined to a) never go through this difficult assay again, b) get the money to buy an HPLC, and c) switch to studying GSH synthesis instead of focusing on its oxidation/reduction. In contrast, Helmut’s

Address correspondence to: Henry Jay Forman, Davis School of Gerontology, University of Southern California, 3715 McClintock Avenue, Los Angeles, California 90089-0191, Tel 209-658-2817, peroxideman@gmail.com.

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work on GSH has been in the area of using GSH to combat oxidative stress as will be described below. That isn't the only difference between Helmut and me (see Fig. 1 for the long and short of that).

We often hear about glutathione being the most abundant antioxidant in cells at 1–10 mM (4), but what may be even more remarkable is that it is the most written about antioxidant! Table 1 shows the number of citations about major antioxidants as of March 31, 2015. Therefore, as the subject is so well known, the introduction to this marvelous molecule will be brief.

The discovery of GSH, identification of its structure as L- γ -glutamyl-L-cysteinyl-glycine, and early recognition of its functions were reviewed on the hundredth anniversary of its discovery by Meister (sometimes fondly referred to as the godfather of glutathione) in 1988 (5). The functions of GSH are primarily in reactions that are protective of cells and organisms. GSH is used as an enzyme substrate for the glutathione peroxidases (GPXs), where it is the reductant of hydroperoxides, and by glutathione S-transferases (GSTs), which conjugate GSH to electrophiles. One use of GSH that is not protective is the conjugation of GSH to leukotriene A₄ by a GST, which produces leukotriene C₄, the slow reacting substance of anaphylaxis. While the rate of its non-enzymatic reduction of hydroperoxides is insignificant compared with that for GPXs (6,7), GSH reacts non-enzymatically with several electrophiles at rates approaching those for the GSTs (8). For more on the roles of GSH, GPXs and GSTs in cellular defense I refer readers to the many excellent reviews on the subject that can be found easily while I escape the ire of those whose articles I would have inadvertently left out by selecting a few from the nearly 3000 review articles in a Scopus search for “glutathione and (peroxidase or GST).”

According to the Leopold Flohé (see his article in this issue), Helmut Sies began his involvement with the use of GSH in removing hydroperoxides as a skeptic. But, like many converts, he became a fanatic, revealing many of the fundamental aspects of GSH metabolism. As mentioned above, Helmut has published 173 articles that concern GSH. His research has also focused on how selenium plays a vital role in this process and how seleno compounds can mimic the action of the glutathione peroxidases. As Flohé, who played a major role in the early work, described Helmut's contributions in this area, I have refrained from repetitiveness except for pointing out that Helmut's work on the oxidation and reduction of GSH as a critical part of antioxidant defense led to his concept of “oxidative stress,” perhaps his more significant scientific contribution (9).

Synthesis of GSH

Although cells have relatively high concentrations of GSH, an increase in synthesis of GSH is clearly part of the adaptive response to oxidative stress. This involves three pathways (Fig 2). One is an increase in the ability to reduce GSSG to GSH through the action of GSSG reductase (10,11). The second is an increase in *de novo* GSH synthesis through induction of glutamate cysteine ligase (GCL), originally called γ -glutamylcysteine synthetase (see below for discussion). The third is an increase in the enzyme γ -glutamyl transpeptidase (GGT), an enzyme that is on the outer surface of cells and catalyzes the transfer of the γ -glutamyl

moiety of GSH to amino acids (12). The favored acceptor is cystine, the disulfide of cysteine, used to produce γ -glutamylcystine, which is taken up by cells and reduced to γ -glutamylcysteine, the same product as formed in *de novo* GSH synthesis. The use of cystine and extracellular GSH to form intracellular γ -glutamylcysteine is called the scavenger pathway (13), which can also recover the glutamate from γ -glutamylamino acids through its oxidation to 5-oxoproline and re-reduction (14). Work in my lab has largely focused on regulation of the induction of GCL and GGT, which will be briefly summarized here.

GCL regulation occurs both by allosteric regulation of its enzymatic activity and through altered expression of its catalytic (GCLC) and modulatory (GCLM) subunits. GCLC, the larger subunit that has low catalytic activity in the absence of GCLM, is inhibited by GSH in classic feedback inhibition (15). The smaller GCLM modulates catalytic activity of GCLC by reducing feedback inhibition by GSH and decreases the K_M for glutamate (16). Although the two subunits appear to form a 1:1 complex, elevated GCLM/GCLC expression raises the relative GCL activity suggesting that under physiological conditions not all of the GCLC may have GCLM bound to it so that while increased GCLC expression will elevate GSH and increasing GCLM/GCLC will increase GSH further (17). On the other hand HIV-Tat protein suppresses GSH by decreasing GCLM expression (18). The effects of both GCL subunits on enzymatic activity are also regulated by phosphorylation (19).

GCL expression is regulated by several mechanisms. Electrophiles including hydroperoxides increase the transcription of both GCLC and GCLM and possibly the stability of the mRNAs as well (20,21) (see review by Lu (22)). Although it was recognized that low concentrations of electrophiles could increase GSH concentration after an initial drop due to oxidation and/or conjugation (23,24). My lab was interested in how GSH countered quinone toxicity and we fortunately discovered about that time that quinones increased the transcription of GCLC (Shi et al., 1994; Shi et al., 1994). We and others then demonstrated that GCLM was also transcriptionally upregulated by a number of electrophiles (Rahman et al., 1996; Tian et al., 1997; Galloway and McLellan, 1998; Liu et al., 1998; Moellering et al., 1999; Wild and Mulcahy, 1999). Mulcahy's group demonstrated that the electrophile response elements, EpRE (aka ARE or antioxidant response element), were present in both the GCLC and GCLM promoter sequences (25,26); however, we also demonstrated that activation of the TRE (AP-1 binding) element was also critical in induction of both GCLC and GCLM (27). During the next twenty years many labs contributed to understanding the signal transduction involved in electrophilic activation of AP-1 and Nrf2, the transcription factor that binds to EpRE. But, as the focus here is on GSH, I'll leave further discussion of GCL induction at this point.

My laboratory's first foray into molecular biology involved the discovery that transfecting GGT into cells increased its effective use of the scavenger pathway described above (28). Shortly, after that, we discovered that GGT could be induced by quinones (12). Over the next several years we found that human GGT transcription was regulated by both the EpRE and TRE elements as summarized in (29).

Transport of GSH and GSSG

Both GSH and GSSG are transported out of cells. Hepatocytes transport GSH, which can then be used to supply cysteine for GSH synthesis in other organs (Anderson et al., 1980). Indeed, Helmut showed that plasma GSH is very low concentration because of its use by other cells to synthesize GSH (30). As described above, the scavenger pathway uses GGT, which transfers glutamate from GSH to other amino acids while releasing cysteinylglycine. Dipeptidases on the cell surface hydrolyze cysteinylglycine releasing glycine cysteine and glycine, which along with γ -glutamyl amino acids are taken up by cells (31–33).

In his early work on glutathione, Helmut, demonstrated that GSSG was exported by isolated perfused liver in response to mitochondrial H_2O_2 production (34,35). I refer again to the article in this issue by Flohe', which describes the circumstances of this discovery. Helmut's early work on GSSG export was done at the University of Pennsylvania, which he left about the time I arrived there. At Penn, I collaborated with Aron Fisher on isolated perfused lungs in which we demonstrated that paraquat induced GSSG release was decreased by selenium deficiency (36). Thus, Helmut influenced another aspect of my work in this field.

Several years later, my laboratory showed that the secretion of GSH from the apical surface of epithelial cells from the trachea and bronchi of lungs was regulated by CFTR, a chloride channel that is missing or defective in cystic fibrosis (37). While others claimed that CFTR could transport GSH, which we did not observe, we demonstrated that a non-GSH-transporting artificial chloride channel was able to restore GSH secretion when added to epithelial cells derived from a CF patient (38).

Glutathionylation of proteins – protection against greater oxidation and post-translational modification

Following the observations by Helmut that protein mixed disulfides (conjugates of protein cysteine to GSH) increased in oxidative stress (39), we reported the predicted increase in selenium deficiency (40). Helmut had suggested that the formation of the mixed disulfides would protect protein cysteines from greater oxidation to sulfinic or sulfonic acids, which are not readily reduced, and demonstrated that formation of mixed disulfides with one protein in liver was predominant under oxidizing conditions (41). But, earlier, he had demonstrated that significant metabolic changes occurred in response to hormonal stimulation that were associated with mixed disulfide formation (42). In other words, Helmut demonstrated an underlying principle of redox signaling well before it was called that or associated with non-metabolic signaling.

My laboratory has spent the past thirty years investigating the relationship of oxidant production, primarily H_2O_2 and 4-hydroxy-2-nonenal to signal transduction, first as inhibitors and then as second messengers. An early publication in the redox signaling area was the demonstration that H_2O_2 made by stimulated macrophages activated NF- κ B signaling (43). At that time, phagocytes were the only cells known to produce H_2O_2 upon stimulation of an NADPH oxidase rather than as a byproduct of metabolism). Many other studies had previously demonstrated signaling by the addition of exogenous H_2O_2 . So, our

report was looked upon as a curiosity of phagocytes. Many signaling pathways have subsequently been demonstrated to be activated by endogenously produced H_2O_2 , particularly after the discovery by Lambeth of the multiple and ubiquitous NADPH oxidases (reviewed in (44)). Regardless, how H_2O_2 actually functioned as a second messenger was not revealed until it became apparent that some of the targets formed protein mixed disulfides! The mechanism of formation has been proposed by some to be an intermediate formation of a sulfenic (-SOH) acid form by non-enzymatic oxidation, although the kinetics in the presence of millimolar competing GSH do not support that argument. Nonetheless, mechanisms for the formation of the mixed disulfides have been suggested that are kinetically and physiologically feasible with a few demonstrated examples (for reviews see (45,46). Regardless of the actual mechanism, Helmut Sies observation of the formation of protein mixed disulfides by hormonal stimulation (42) was probably the first demonstration of the mechanism for much of redox signaling.

Writing this a few months short of following Helmut's footsteps into the role of Editor-in-Chief of Archives of Biochemistry, I am very pleased to state that I have had first hand knowledge and am very appreciative of how his outstanding pioneering work has paved the way for others to follow.

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Figure 1.
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Figure 2.

The interrelationships of glutathione synthesis, transport, and use as in intracellular detoxification. Abbreviations: GSH, glutathione GSSG, glutathione disulfide; GGT, γ-glutamyl transpeptidase; GCL, glutamate cysteine ligase; GS, glutathione synthetase; GST, glutathione S-transferase; GR, glutathione reductase; GPx, glutathione peroxidase; ROOH, lipid hydroperoxide; ROH, lipid alcohol; X, electrophile; GSX, glutathione conjugate to X

Table 1

Glutathione, the most commonly written about cellular antioxidant

Search term(s)	Scopus search	Pubmed search
Glutathione	158,143	118,785
Cysteine	139,205	114,255
Ascorbic or vitamin C	134,252	53,704
Alpha-tocopherol or vitamin E	88,685	41,164
NADPH	52,945	64,721
NADH	38,259	66,456
Thioredoxin	11,729	9,164
S-adenosylmethionine	10,990	7,826