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Glutathione – From antioxidant to post-translational modifier

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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.

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1. 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 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),

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Fig. 1. Henry Jay Forman and Helmut Sies 2014 Kyoto, Japan.

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	9164
S-adenosylmethionine	10,990	7826

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].

2. 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].

3. 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

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