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The changing faces of glutathione, a cellular protagonist

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Abstract

Glutathione (GSH) has been described for a long time just as a defensive reagent against the action of toxic xenobiotics (drugs, pollutants, carcinogens). As a prototype antioxidant, it has been involved in cell protection from the noxious effect of excess oxidant stress, both directly and as a cofactor of glutathione peroxidases. In addition, it has long been known that GSH is capable of forming disulfide bonds with cysteine residues of proteins, and the relevance of this mechanism (''S-glutathionylation'') in regulation of protein function is currently receiving confirmation in a series of research lines. Rather paradoxically, however, recent studies have also highlighted the ability of GSH—and notably of its catabolites—to promote oxidative processes, by participating in metal ion-mediated reactions eventually leading to formation of reactive oxygen species and free radicals. A crucial role in these phenomena is played by membrane bound γ-glutamyltransferase activity. The significance of GSH as a major factor in regulation of cell life, proliferation, and death, should be regarded as the integrated result of all these roles it can play.

Keywords: Glutathione; Glutathione-dependent enzymes; Thiols; Detoxication; Cell regulation

1. Introduction

Since the first hint of the existence of an ''organic stuff'' related to the metabolism of sulfur (de Rey Pailhade, 1888; quoted in [1]), 115 years of biomedical research have documented innumerable situations in which GSH—a tripeptide consisting of glycine, cysteine and glutamic acid—participates in essential aspects of cellular homeostasis. GSH has revealed different aspects of its function every time the perspective on it has been moved—and still the whole story has not been told yet. Quite a number of excellent reviews are available dealing with GSH biochemistry and pathophysiology. To these the reader should refer in order to appraise the details of the matter. The present commentary is meant to give an overview of the different perspectives that presently animate GSH research.

2. "*Antitoxic glutathione*"—nucleophilic reactions mediated by GSH and GS-S-transferases

GSH is among the most efficient tools that cells can exploit in detoxification of drugs and xenobiotics in general. GSH in fact is both a nucleophile and a reductant, and can therefore react with electrophilic or oxidizing species before the latter interact with more critical cellular constituents such as nucleic acids and proteins. A number of studies are present in the literature dealing with the protection offered by adequate GSH levels to cellular macromolecules against the noxious effects of a wide array of toxic agents (reviewed in [2]). Both chemical and enzymatic reactions are involved in the ''antitoxic'' function of GSH, as it serves as a cofactor in conjugation reactions mediated by GS-S-transferases. Several instances exist, however, in which GSH conjugation lacks any protective connotation, as it can give rise to the formation of compounds that are at least as reactive as the parent electrophiles. Conjugation e.g. may be reversible, with subsequent release of the electrophile, as in the case of benzyl isothiocyanate. The conjugate then serves just as a storage form of the

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electrophile. Alternatively, GSH conjugation may cause the formation of a toxic derivative. The conjugate itself may be reactive (e.g. 2-bromoethylglutathione, reacting via an episulfonium ion), or can undergo further metabolism originating toxic species (reviewed in [3]).

There has been a blossoming of studies on the genetic arrangement of the GS-S-transferase family, which includes soluble as well as membrane bound members provided with different—although sometimes overlapping—substrate specificity. Much of the attention currently dedicated to GS-S-transferases is prompted by the possibility that these activities may act as determinants of the individual response to chemical carcinogens and antineoplastic drugs. These important aspects have been thoroughly treated in a recent review [4].

GSH-adducts formed after conjugation of electrophiles are then actively secreted from the cell, and the existence of various cellular systems for the efflux of GS-conjugates again pertains to drug resistance of tumors [2]. Conjugation and efflux can eventually result in the depletion of cellular GSH, and when the latter is severe, cellular defence based on GSH conjugation is impaired. Electrophiles would then freely exert their injuring action by interacting with critical macromolecular targets within the cell. Cell injury induced by electrophiles was thus long believed to be the mere result of alkylation of cellular macromolecules by their reactive metabolites. Several studies, however, highlighted that, in some instances, most of the cell injury occurring after GSH depletion may actually depend on the onset of extensive, uncontrolled oxidative processes such as lipid peroxidation. The basis for toxicity of several electrophiles might thus lie in their ability to suppress GSH-based antioxidant defenses of the cell, rather than in a supposed damage caused by their covalent binding to cellular constituents [5–7].

The significance of GSTs seems to extend farther than GSH conjugation of xenobiotics. Other reasons may have concurred to the evolutionary conservation of these protein structures. GS-S-transferase Pi (GSTp) has been found, e.g. to form macromolecular complexes with Jun N-terminal kinase (JNK), thus acting as a JNK inhibitor capable of preventing c-Jun phosphorylation *in vitro* [8].

3. "*Antioxidant glutathione*"—GSH as a cofactor for GSH-peroxidases and other enzymes active in cell defense against prooxidants

Many of the drugs and xenobiotics detoxified through conjugation with GSH are oxidants in nature, and are able to produce oxidative injury to cells. In such a sense, GSH and GS-S-transferase-mediated conjugation represent a major aspect of the ''antioxidant'' function of GSH. In addition, several GS-S-transferases are actually able to act as peroxidases, by reducing hydroperoxides in a GSH-dependent manner. GSH is the cofactor for the numerous members of the GSH-peroxidase families. Like GSTs, GSH-peroxidases include both soluble and membrane-bound members, and there are selenium-dependent and selenium-independent (GST) enzymes. The GSH peroxidase system is critical as a defense mechanism against potentially toxic hydrogen peroxide and other peroxides, including lipid hydroperoxides [2]. During GSH-peroxidase-mediated detoxication of peroxides, glutathione disulfide, GSSG ('oxidized GSH') is formed. It has been shown that GSSG is capable of effecting thiol-exchange reactions on thiol residues of proteins, leading to the formation of mixed disulfides. The cellular GSH pool can be regenerated from GSSG via the NADPH-dependent enzyme GSSG reductase. In consideration of its role in maintaining cellular levels of NADPH, glucose 6-phosphate dehydrogenase can be thus regarded as an ''antioxidant'' enzyme [9].

The antioxidant function of GSH is also exerted through its participation to other, primary antioxidant systems of the cell [10–13]. Several GSH-dependent enzymes, e.g. have been described, provided with dehydroascorbate reductase activity [14,15]. In addition, a novel GSH-dependent dehydroascorbate reductase has been identified and characterized [16,17]. The physiological significance of these enzymes lies in their ability to regenerate ascorbate from the oxidation product, dehydroascorbate, by using glutathione as an electron donor. Due to its properties (presence in adequate amounts within the cells; capability to react with a variety of free radicals; suitability of regeneration) ascorbic acid can be viewed as the 'perfect antioxidant' for the cells of nearly all aerobic organisms [18]. Following the reaction with free radicals, ascorbate undergoes one-electron oxidation with formation of ascorbyl radical; the latter—either by dismutation or by further oxidation—is converted to the divalent oxidation product dehydroascorbate. A key mechanism to maintain adequate cellular levels of ascorbate—independently of *de novo* synthesis and dietary supply—involves the reduction of this oxidized form back to reduced ascorbic acid (''*ascorbate recycling*''). GSH-dependent dehydroascorbate reductase could represent a major cellular tool in order to maintain steady-state concentrations of ascorbic acid in conditions of accelerated oxidation.

4. "*Modulator glutathione*"—the significance of protein S-glutathionylation

A number of functionally critical proteins within the cell possess accessible cysteine residues, liable to undergo redox changes depending on variations of the intra- as well as extracellular conditions. The antioxidant action of GSH (and/or, in selected circumstances, its GGT-mediated 'prooxidant' action) are capable of affecting the redox status of such critical thiols in proteins, which makes of cellular GSH a crucial modulating factor for an ever

increasing number of proteins (membrane channels and transporters, receptors, protein kinases and phosphatases, transducers, etc.). Interactions of this kind have also been documented with transcription factors, which provides GSH with a further pathophysiological role in modulation of gene expression [19,20]. Moreover, GSH itself can become directly bound with (cysteine) thiols comprised in polypeptidic chains, forming so-called 'mixed' (protein–nonprotein) disulfide bridges. This can occur following prior two- or four-electron oxidation of protein SH groups (e.g. to sulfenic acids), or as the result of thiol/disulfide exchange in the presence of increased cell levels of GSSG [21].

The potential importance of these aspects had been envisaged in earlier studies, and has fostered speculation during decades [22–24]. Experimental evidence started to accumulate recently, documenting that the binding of GSH to proteins—a process termed '*protein S-glutathionylation*'—occurs in a number of physiologically relevant situations, where it can produce discrete modulatory effects on protein function. An increasing number of targets have been identified, including membrane as well as cytosolic proteins [25,26]. Remarkably, S-glutathionylation can affect members of signal transduction chains involved in cell proliferation (e.g. H-ras: [27]; T cell $p59^{fyn}$ kinase: [28]; PTP1B phosphatase: [29]; c-*jun*: [30]; NF-kB/p50: [31]). Proteins playing crucial roles in the apoptotic machinery are also likely to be involved. Primary structure of caspase-3 is related with HIV-1 protease, and S-glutathionylation of the latter was shown to occur [32]. Critical cysteine residues are comprised in DNA-binding domain of p53, and at least four of them are available to oxidative modifications with specific effects on DNA binding ability [33].

Several enzyme activities are able to effect protein "dethiolation", i.e. the detachment of glutathione from mixed disulfides, and the implications of these activities in cell physiology represent one of the developing edges of the field [34]. Conceivably, the cysteinyl-glycine originating at sites of GSH catabolism by membrane γ-glutamyltransferase (GGT) might as well concur to the formation of disulfide bridges on proteins, with allegedly comparable functional effects. This is an aspect currently under investigation in our laboratories.

5. "*Prooxidant glutathione*"—a result of the metal-reducing ability of thiols

GSH is able to bind metal cations, a feature likely exploited by the cell during transport and delivery of metals [35]. Interactions of GSH with metal ions can however be more complex, with important bearings on the redox environment of the cell. Thiol compounds—especially when dissociated to their thiolate anion ($R-S^-$) forms—can in fact effect the reduction of metal cations, e.g. iron, copper. Electrons can be then transferred in turn from metal ions to molecular oxygen, thus generating superoxide anions which will easily dismutate to the strong prooxidant hydrogen peroxide. The sequence—a true "redox cycling" [36] of metals—can proceed with even minimal concentrations of metal ions as long as electron donors (thiols) and acceptor (molecular oxygen) are available in the system. In this way, paradoxically, the 'reducing' ability of thiols is finally turned into overall 'oxidizing' effects (e.g. lipid peroxidation [37,38]). The efficiency of individual thiol compounds in metal ion reduction is determined by the relative pK_a's of their thiols, i.e. by the ease of dissociation of their SH groups to the corresponding thiolate anions. GSH itself is rather less efficient than other thiols, an aspect which can be explained by the vicinity of the SH group of cysteine to the α-carboxyl group of glutamic acid [39]. The removal of glutamic acid in fact produces a marked increase in the metal-reducing ability, resulting in a promotion of oxidative processes [38,39].

The cleavage of the γ-glutamyl bond in GSH is physiologically effected (extracellularly) by membrane-bound GGT, the sole enzyme capable of doing it, expressed at various levels in cells and tissues. The possibility thus exists that sites of GGT activity may act as sites of promotion of metal ion reduction and redox cycling, with consequent stimulation of oxidative processes. Our studies have indeed verified this hypothesis in a series of conditions, including liver carcinogenesis, human atherosclerosis, kidney ischemia, GGT-expressing cancer cells (reviewed in [40]). Importantly, it was also established that GGT/GSH-mediated prooxidant reactions can "*oxidatively*" participate to redox modulation of both proliferative and apoptotic signals, which prompted the definition of "*GSH catabolism as a signaling mechanism*" [41]. These findings enrich the pivotal position occupied by GSH in cell redox regulation with aspects of complexity, which can help explain peculiar observations made in selected experimental conditions.

On the other hand, being GGT-derived cysteinyl-glycine a nucleophile even stronger than parent GSH, sites of GSH catabolism may also function as sites where conjugation with electrophilic agents is efficiently promoted. In this way, cells expressing sufficient GGT activity at their surface might be able to effect sort of an "*extracellular detoxication*" of electrophilic drugs. Our recent studies have confirmed that these mechanisms indeed identify membrane-bound GGT activity as a factor of drug resistance, in normal as well as cancer cells [42,43], a finding that opens novel perspectives for future studies in the field of cancer chemotherapeutics.

One additional feature of the "prooxidant" role potentially played by GSH lies in its capability of combining with the free radical nitric oxide ($^{\bullet}NO$), to form S-nitrosoglutathione (GSNO), actually serving as a transport form for this potent prooxidant involved in several pathophysiological conditions.

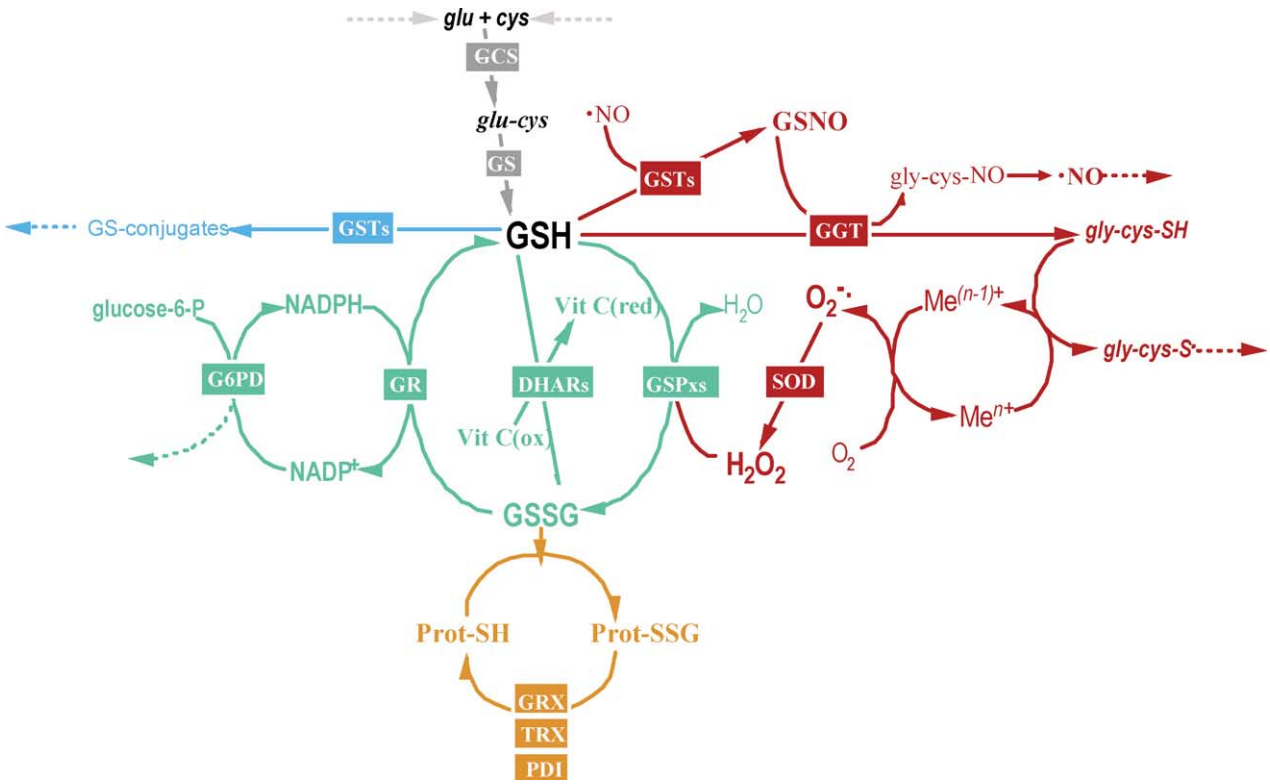

Fig. 1. Outline of the interrelations connecting the various roles played by GSH in cellular homeostasis: *antitoxic* (blue), *antioxidant* (green), *prooxidant* (red), *modulator* (yellow). Abbreviations: γ-GCS, gamma-glutamyl-cysteine synthetase; DHARs, dehydroascorbate reductases; G6PD, glucose 6-phosphate dehydrogenase; GPxs, glutathione peroxidases; GR, glutathione reductase; GRX, glutaredoxin; GS, glutathione synthetase; GSNO, S-nitrosoglutathione; GSTs, glutathione S-transferases; Me, metal; PDI, protein disulfide isomerase; SOD, superoxide dismutase; TRX, thioredoxin.

6. Conclusion

GSH is a true protagonist in the fascinating drama of cell regulation. All the aspects outlined above (summarized in a graphic form in Fig. 1) should be carefully evaluated, when considering the participation of GSH in primary cellular processes such as gene expression, cell proliferation or apoptosis [20,44,45]. Is it to be expected that even more faces, more roles of GSH may emerge from future research? At least one novel, redox-unrelated aspect was recently described and deserves quotation here: it has been reported that binding sites provided with true specificity for GSH exist in the central nervous system, and this satisfies the main requisite for considering GSH as a neuromediator [46]. Indeed, the significance of GSH and reactions related to its transport and metabolism—after decades of fruitful research—still holds aspects to investigate, plots to unveil.

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