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Glutathione catabolism as a signaling mechanism

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Abstract

Glutathione (GSH) is the main intracellular thiol antioxidant, and as such participates in a number of cellular antitoxic and defensive functions. Nevertheless, *non-antioxidant* functions of GSH have also been decribed, e.g. in modulation of cell proliferation and immune response. Recent studies from our and other laboratories have provided evidence for a third functional aspect of GSH, i.e. the *prooxidant* roles played by molecular species originating during its catabolism by the membrane ectoenzyme γ-glutamyl transpeptidase (GGT). The reduction of metal ions effected by GSH catabolites is capable to induce redox cycling processes leading to the production of reactive oxygen species (superoxide, hydrogen peroxide), as well as of other free radicals. Through the action of these reactive compounds, GSH catabolism can ultimately lead to oxidative modifications on a variety of molecular targets, involving oxidation and/or S-thiolation of protein thiol groups in the first place. Modulating effects of this kind have been observed on several important, redox-sensitive components of the signal transduction chains, such as cell surface receptors, protein phosphatase activities and transcription factors. Against this background, the prooxidant reactions induced by GSH catabolism appear to represent a novel, as yet unrecognized mechanism for modulation of cellular signal transduction.

Keywords: Glutathione; γ-Glutamyltransferase; Hydrogen peroxide; Protein thiols; Signal transduction; NF-κB; AP-1

Oxidation/reduction reactions are a primary mechanism for regulation of numerous important functions of the cell. A number of biomolecules involved in signal transduction and regulation of gene expression are sensitive to prooxidants, e.g. reactive oxygen species (ROS), at concentrations considerably lower than those capable to induce oxidative injury. Prooxidants thus can no longer be regarded as merely offensive species, and similarly, the physiological role of some established ''antioxidants'' also is in need of careful reconsideration. Glutathione (GSH)—perhaps the best known cellular antioxidant—appears an ideal candidate in this perspective. The *antioxidant* role of GSH is readily apparent in detoxification of electrophilic/oxidizing drugs and protection from lipid peroxidation. Nevertheless, *non-antioxidant* functions of GSH have been decribed, e.g. in modulation of signal transduction, cell proliferation and immune response. To complete the picture, recent studies point to *prooxidant* effects of (extracellular) GSH, which can ensue from its catabolism by the membrane ecto-enzyme γ-glutamyltransferase (GGT). It has in fact been documented in our and other laboratories that prooxidant species (superoxide, H_2O_2, thiyl radicals) are produced during GSH catabolism, as a result of the interaction of GSH metabolites—cysteinyl-glycine in the first place—with trace levels of iron ions present in the cell environment. The interaction of these GSH/GGT-derived prooxidants several intra- and extracellular targets is responsible for appreciable modulatory effects on the signal transduction chains.

1. GSH, GGT and iron reduction

γ-Glutamyl transpeptidase (E.C. 2.3.2.2) is normally found in serum, and is expressed by a wide range of normal cell types [1,2] as well as in a number of neoplastic cell

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Abbreviations: AP-1, activator protein-1; AT-125, acivicin; ECL, enhanced chemiluminescence; EMSA, electrophoresis mobility shift assay; GGT, γ-glutamyl transpeptidase; gly-gly, glycyl-glycine; GSH, glutathione; GSH-DME, glutathione dimethyl ester; NF-κB, nuclear factor-κB; PARP, poly(ADP-ribose) polymerase; PP, protein phosphatase; ROS, reactive oxygen species; TNFR1, tumor necrosis factor-α receptor 1.

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lines [3] and human spontaneous tumors (reviewed in [4]). GGT catalyzes the first step in the degradation of extracellular GSH, i.e. the hydrolysis of the γ-glutamyl bond between glutamate and cysteine [5]. In so doing GGT releases cysteinyl-glycine, which is subsequently cleaved to cysteine and glycine by plasma membrane dipeptidase activities [5].

Stark *et al.* [6] first proposed that the catabolism of GSH can play a prooxidant role in selected conditions. These authors suggested that the GGT-mediated cleavage of GSH—allegedly through the generation of the more reactive thiol cysteinyl-glycine—could cause the reduction of ferric iron Fe(III) to ferrous Fe(II), thus starting an iron redox-cycling process liable to result in the production of ROS and stimulation of oxidative reactions. GGT was thus shown to stimulate a GSH-dependent lipid peroxidation in model systems involving Fe(III) complexes as redox catalysts and purified linoleic acid peroxidizable substrate [6]. In these systems, the "prooxidant" effect of GGT was attributed to the formation of cysteinyl glycine and cysteine, which reduce Fe(III) more efficiently than does GSH [7,8]. Indeed, in systems including ADP–Fe(III) complexes GSH itself can reduce some iron, but the reaction rate increases significantly when GGT is included to remove its γ-glutamate residue (Fig. 1A). Maximal rates of ADP–Fe(III) reduction are observed after co-incubation of GSH and GGT with glycyl-glycine (gly-gly), which accelerates the enzymatic hydrolysis of GSH by serving as a terminal acceptor of γ-glutamyl moieties. *In vivo*, this function is assisted by a number of acceptor aminoacids [5]. Inasmuch as neither GGT nor GGT plus gly-gly can reduce ADP–Fe(III) directly, these results show that GSH-enhanced iron reduction is enhanced by enzymatic removal of γ-glutamate. This effect was observed over a broad range of GGT activities and GSH concentrations [9].

Previous studies had shown that the pK_a of the cysteinyl-glycine thiol is significantly lower than that of GSH (6.4 vs. 8.56, respectively) [10]. The ability of GGT to enhance iron reduction by GSH might therefore reflect the formation of cysteinyl-glycine, bearing a thiol moiety which dissociates more rapidly at near-neutral pH, and can thus redox-couple with Fe(III). In agreement with this possibility, cysteinyl-glycine was found to reduce ADP-chelated Fe(III) more effectively than did GSH, forming Fe(II) to the same extent as observed with GSH plus GGT (Fig. 1B). Similar results were obtained with cysteine, that is the product of cysteinyl-glycine hydrolysis by membrane-bound dipeptidase (Fig. 1B).

According to Spear and Aust [8] GSH-dependent iron reduction might be limited by the chelating properties of the α-carboxyl group of γ-glutamate, affecting sterical and redox interactions of the cysteine thiol with Fe(III). To test this hypothesis, GSH dimethyl ester (GSH-DME)—having the glutamate moiety methylated in conjunction with the carboxyl group of glycine, was prepared. As also shown in Fig. 1B, GSH-DME was more effective than GSH in reducing ADP–Fe(III), yielding the same Fe(II) as formed by cysteinyl-glycine, cysteine, or a combination of GSH with GGT. Considering that the thiol moieties of GSH and GSH-DME share very similar pK_a [8], we concluded that ADP–iron reduction was indeed limited by the chelating properties of γ-glutamate, affecting electron transfer from the juxtaposed cysteine thiol to Fe(III) [9].

Redox cycling of iron is a recognized factor in initiation of lipid peroxidation [11]. Accordingly, GSH/GGT-dependent iron reduction was repeatedly shown to result in the promotion of lipid peroxidation, in chemically induced preneoplastic lesions of rat liver [12], in rat liver microsomes and isolated hepatocytes [13], and in isolated human plasma LDL lipoproteins [9].

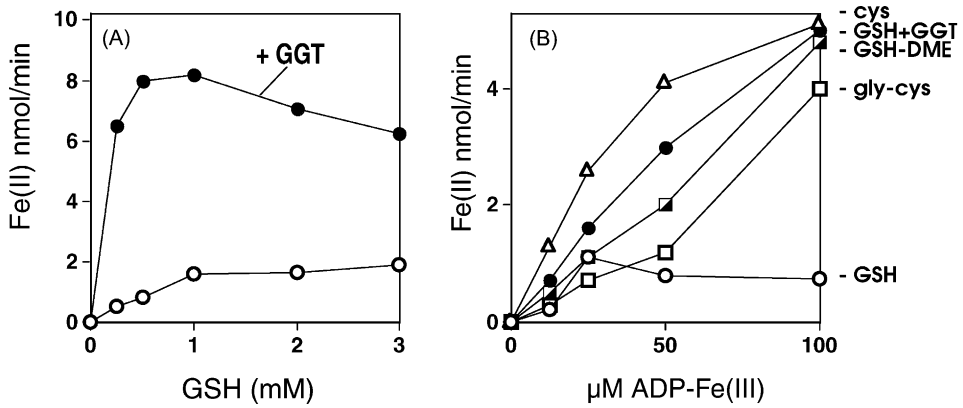

Fig. 1. (A) Effects of GGT on GSH-dependent ADP–Fe(III) reduction. Incubations (1 mL final volume) contained ADP–Fe(III) (1 mM chelator–0.1 mM $FeCl_3$), glycyl-glycine (gly-gly) (20 mM) for stimulation of GGT activity, and bathophenanthroline disulfonate (0.25 mM) for determination of Fe(II) produced, in PBS, pH 7.4, 37°. Reactions were started by adding GSH (2 mM) and increasing amounts of GGT. Values are taken from representative experiments. (B) ADP–Fe(III) reduction by GSH-DME in comparison with GSH, GSH plus GGT or GSH hydrolysis products. Incubations (1 mL final volume) contained increasing amounts of ADP–Fe(III) in PBS as above. Reactions were started by adding 1 mM cysteinyl-glycine, cysteine, GSH or GSH-DME. Where indicated, GSH was co-incubated with GGT (200 mU) and gly-gly (20 mM). Values are those determined at 2 hr and are taken from representative experiments. Data from [9], modified.

Table 1
The inhibition of GGT activity reveals a net GSH efflux from U937 histiocytoma cells

Sample	GSH in supernatant (pmol/10^6 cells/hr)
Control	75 ± 4
Acivicin 15 μM	259 ± 3*
Acivicin 150 μM	444 ± 3*

After 3 hr of GGT inhibition with acivicin in RPMI-1640 medium, cells were washed and resuspended in PBS; the GSH content in the extracellular medium was measured after 1 hr.
*$P < 0.001$, compared to control value. Data from [21], modified.

2. GSH/GGT-dependent prooxidants and the redox status of protein thiols

By cleaving the γ-glutamyl bond in GSH, GGT provides the first step in catabolism of extracellular GSH. With the contribution of membrane dipeptidase activities, precursor aminoacids glycine and cysteine are then released, which can cross the plasma membrane and are re-utilized for intracellular GSH synthesis [14]. Since it has been documented that a continuous efflux of GSH occurs from a number of cell types through specific out-transporters [15], it is conceivable that a major function of GGT ectoactivity could be the salvage of extracellular GSH which would otherwise be lost from the cell. GGT would thus play the main role in sort of a "*GSH cycling*" at the plasma membrane [16], with the apparent task of preserving cellular levels of GSH [17].

This view, however, may not always be sufficient to interpret experimental evidence, as in some instances GGT activity can be dissociated from cellular supply of GSH. Table 1 reports data obtained with U937 histiocytoma cells. Indeed, the inhibition of GGT in these cells reveals the presence of a continuous GSH efflux, since significant amounts of GSH are accumulated in the extracellular medium following treatment with the non-competitive GGT inhibitor acivicin. Nevertheless, as shown in Table 2, GGT inhibition does not result in any decrease of intracellular GSH content, pointing to the possibility that—at least in this cell type—GGT-mediated cleavage of extracellular GSH may be not essential for the maintainance of intracellular GSH levels.

Table 2
Intracellular GSH levels in U937 histiocytoma cells following the inhibition of GGT activity

Sample	Incubation time (hr)	GSH content (nmol/mg protein)
Control	24	23.4 ± 1.3
Acivicin 15 μM		25.8 ± 1.7
Acivicin 150 μM		25.5 ± 2.9
Control	48	22.1 ± 1.1
Acivicin 15 μM		25.3 ± 1.5
Acivicin 150 μM		29.9 ± 1.5

Results are means ± SEM from three separate experiments. Data from [21], modified.

Are there additional functions of GGT, unrelated with cellular supply of GSH? In favor of such view are the results of recent research, focused on the effects produced by GGT-mediated GSH catabolism on the *outer* cellular microenvironment. In studies on the relationships of GGT expression with cellular handling of thiols, we found that cell-specific differences in the balance between transport of sulfur aminoacids and membrane GGT activity are reflected in profound differences in the thiol redox status of the extracellular milieu. With melanoma cells not expressing GGT, the extracellular accumulation of substantial amounts of GSH, GSSG and glutathione–cysteine disulfide was observed. In GGT-expressing cells, the same thiols accumulated extracellularly only after inhibition of GGT activity [18].

Thus, in cells expressing GGT at their surface, the continuous cleavage of extracellular GSH creates peculiar conditions favoring the formation of different disulfide species, involving GSH itself as well as its catabolites. A crucial role in these processes is played by thiol-dependent iron reduction and the subsequent production of prooxidants. Redox cycling of iron in fact leads to the production of ROS [19]—i.e. superoxide anion in the first place, from which in turn by dismutation hydrogen peroxide is originated—as well as of thiyl (–S•) radicals; the latter can easily react to form disulfides. The following overall sequence can be envisaged as set into motion by GGT-mediated catabolism of GSH [6] (GC-SH = cysteinyl-glycine):

$$\mathrm{GSH} \xrightarrow{\mathrm{GGT}} \gamma\text{-glutamate} + \mathrm{GC\text{-}SH} \quad (1)$$

$$\mathrm{GC\text{-}SH} \xrightarrow{\mathrm{pH>7.0}} \mathrm{GC\text{-}S^-}\ (\text{thiolate anion}) + \mathrm{H^+} \quad (2)$$

$$\mathrm{GC\text{-}S^-} + \mathrm{Fe^{3+}} \rightarrow \mathrm{GC\text{-}S^\bullet}\ (\text{thiyl radical}) + \mathrm{Fe^{2+}} \quad (3)$$

$$\mathrm{Fe^{2+}} + \mathrm{O_2} \rightarrow \mathrm{Fe^{3+}} + \mathrm{O_2^-}\ (\text{superoxide anion}) \quad (4)$$

$$\mathrm{O_2^-} + \mathrm{H_2O} \rightarrow \mathrm{O_2} + \mathrm{H_2O_2}\ (\text{hydrogen peroxide}) \quad (5)$$

The production of ROS following iron reduction induced by the GGT-mediated catabolism of GSH has been repeatedly documented [4,20–22]. Fig. 2 reports data obtained with U937 histiocytic lymphoma cells, possessing ≅15 mU GGT/mg protein at their surface. The addition of GSH and co-substrate gly-gly to cells results in a sustained production of hydrogen peroxide. The reaction occurs in the extracellular environment, as shown by the fact that catalase—which cannot penetrate cell membrane—can suppress it (Fig. 2A). Generation of H_2O_2 did not take place with cells in which GGT had been irreversibly inhibited by the non-competitive GGT inhibitor acivicin, nor in the presence of the competitive GGT inhibitor, serine–borate complex (Fig. 2B). H_2O_2 production was markedly decreased in the presence of the Vitamin E analogue Trolox C, and was suppressed by addition of

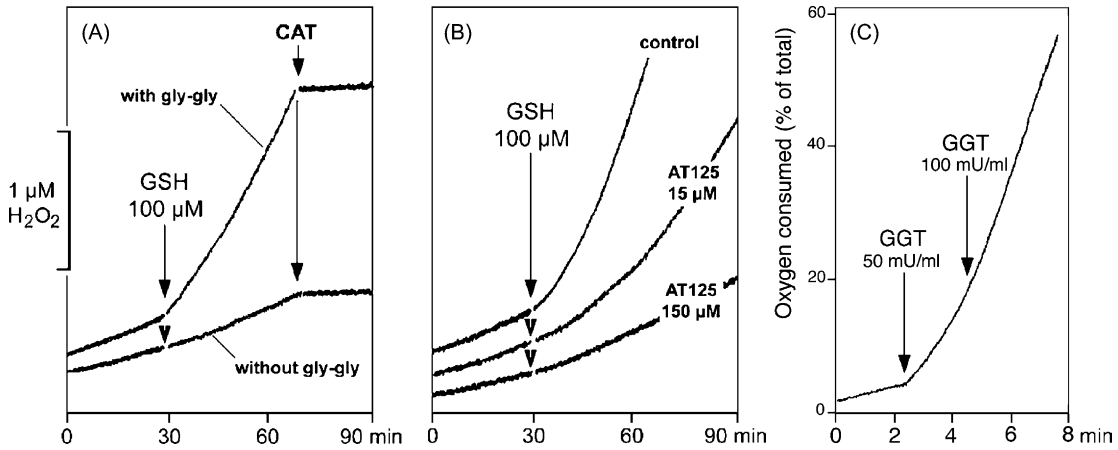

Fig. 2. Production of hydrogen peroxide by U937 histiocytoma cells following the addition of exogenous GSH. H_2O_2 production was measured by fluorimetric monitoring of the horseradish peroxidase (HRP)-catalyzed oxidation of the fluorescent probe *N*-acetyl-3,7-dihydroxyphenoxazine (A6550), which is non-fluorescent and becomes highly fluorescent after oxidation by H_2O_2. (A) The enhancing effect of gly-gly, added in order to stimulate GGT activity of cells, and the suppressing effect of catalase (CAT). (B) The inhibitory effects of increasing concentrations of the non-competitive GGT inhibitor, acivicin (AT-125). (C) The oxymetrical determination of the oxygen consumption induced by GSH catabolism, started by adding purified bovine kidney GGT to an acellular system including GSH (2 mM), gly-gly (200 mM) and Fe(III) (150 μM $FeCl_3$), in PBS, pH 7.4, under continuous stirring at 37°.

low concentrations of the non-cell permeant iron chelator HES–DFO, as well as by addition of a structurally unrelated metal chelator, EDTA [4], thus confirming the involvement of extracellular iron ions in the reaction. As expected, H_2O_2 production could also be directly started by the addition to assay mixture of cysteinyl-glycine, i.e. the metabolite resulting from GGT-mediated cleavage of GSH, although with a relatively low efficiency as judged on a molar basis [4]. As complementary data, Fig. 2C also shows how GGT-mediated GSH catabolism in the presence of Fe(III) is accompanied by a consumption of the oxygen present in the medium.

The availability *in vivo* of iron and other redox-active metals—like copper—in unbound, redox-active form is very restricted, and the question therefore arises whether the described iron-dependent reactions may take place in the normal cell environment. Actually, the release of free iron ions from its storage sites can occur in a whole series of pathophysiological conditions. This is, e.g. the case of iron released from ferritin or haem by the action of superoxide and hydrogen peroxide, two agents produced following the activation of phagocytic cells [23]. Moreover, it has been demonstrated that the reducing power of GSH catabolites originated by GGT is sufficient to effect the reductive release of redox-active iron from transferrin [6,20]. GSH catabolism by GGT could thus represent itself a mechanism capable to increase locally the availability of free iron, which would then catalyze the redox reactions leading to the production of prooxidant species.

Conceivably, a primary target for the action of prooxidants generated extracellularly during GGT activity would be given by thiols of proteins located at the cell surface. The possibility that GSH/GGT-dependent prooxidants could interfere with the redox status of thiol groups contained in proteins at this site was thus investigated. To this aim we developed and validated a procedure for the selective labeling of thiols of cell surface proteins [4]. Indeed, the results obtained revealed the occurrence of a GGT-dependent oxidation of thiol groups in surface proteins of U937 cells. Protein thiol oxidation was in fact increased following stimulation of GGT activity, while the process was prevented after its inhibition. The involvement of hydrogen peroxide in the process is indicated by the fact that protein thiol oxidation was significantly prevented by catalase. Experiments also showed that GGT-dependent decrease in reduced protein thiols was partly due to protein S-thiolation reactions, and that GGT inhibition by acivicin is *per se* sufficient to produce an increase of reduced protein thiols at the cell surface [4]. The latter observation seems to indicate that in GGT-rich cells, surface proteins are continuously exposed to a GGT-dependent oxidant stress, which maintains their thiols partially oxidized. One of the physiological roles of GSH catabolism by GGT could thus lie in its ability to modulate the redox status of cell surface protein thiols.

3. Effects on the cellular proliferation/apoptosis balance

It is widely recognized that prooxidants can play a modulatory role on the transduction of proliferative/apoptotic signals, due to their ability to interact with redox-sensitive regions of growth factor receptors, protein kinases and transcription factors [24–26]. A first indication that prooxidant reactions originating from GSH catabolism could play a role in these processes came from studies with human A2780 ovarian cancer cells, showing that exogenous GSH exerts an antiproliferative action, and that this is an effect of H_2O_2 and thiol oxidation produced by GGT-

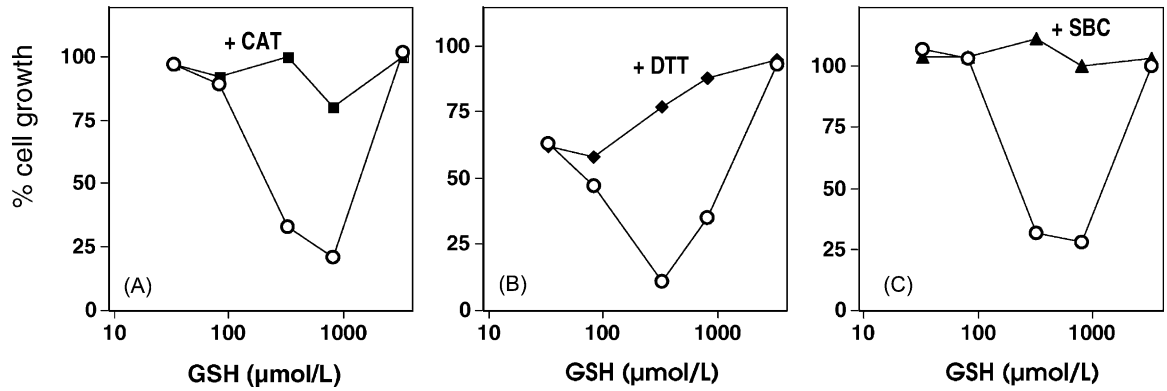

Fig. 3. Mechanisms of GSH-induced growth inhibition in A2780 ovarian carcinoma cells. Determinations were performed by growth-inhibition assay after 1 hr simultaneous exposure to GSH and the individual treatments: (A) suppression of GSH-induced growth inhibition in A2780 ovarian carcinoma cells by catalase (CAT, 100 U/mL); (B) suppression of GSH-induced growth inhibition by dithiothreitol (DTT, 0.1 mM); (C) suppression of GSH-induced growth inhibition by competitive inhibition of GGT activity with serine/boric acid complex (SBC, 1 mM). Data from [27], modified.

mediated extracellular GSH catabolism (Fig. 3). The antiproliferative effect of GSH in fact was reversed by catalase and by dithiothreitol, indicating the occurrence of oxidative phenomena resulting in the impairment of critical cellular thiols. Treatment of cells with hydrogen peroxide also resulted in growth inhibition in A2780 cells. The γ-glutamyl acceptor gly-gly, a cofactor for GGT activity, potentiated the growth-inhibitory effect of GSH, which in contrast was decreased by the GGT inhibitors, serine/boric acid complex and acivicin, indicating that the production of reactive forms of oxygen, hydrogen peroxide in the fist place, was mediated by cysteinyl-glycine produced during GGT-mediated GSH hydrolysis [27].

To remark the complexity of the picture, however, subsequent studies in U937 histiocytic lymphoma cells also showed that a continuous GGT-dependent production of H2O2 can provide tumor cells with a basal, "anti-apoptotic" signal. Previous work had shown that mild oxidative conditions can counteract apoptotic stimuli [28]. Since the inhibition of GGT is a sufficient stimulus for the induction of apoptosis in selected cell lines, we investigated whether this effect might result from the suppression of the mentioned GGT-dependent prooxidant reactions, in the hypothesis that the latter may represent a basal antiapoptotic and proliferative signal for the cell. Experiments with U937 cells showed that: (i) GGT inhibition results in cell growth arrest, and induces cell death and DNA fragmentation with the ladder appearance of apoptosis; (ii) treatment of cells with catalase is able to decrease their proliferative rate; (iii) GGT inhibition (with suppression of H_2O_2 production) results in a down-regulation of poly(ADP-ribose) polymerase (PARP) activity, soon after followed by the proteolytic cleavage of PARP molecule itself, such as that typically induced by caspases [21]. In conclusion, data indicate that the low H_2O_2 levels originating as a by-product during GGT activity are capable to act as sort of a "life signal" in U937 cells, insofar as they can maintain cell proliferation and protect against apoptosis, possibly through an up-regulation of PARP activity [21].

4. Molecular targets in the signal transduction chains

The described effects on the proliferative/apoptotic balance of cells imply the interaction of GSH/GGT-mediated prooxidants with critical targets in the intracellular signal transduction cascade, of which PARP is a first example. Among several redox-sensitive targets, the transcription factor NF-κB is perhaps the best known and studied [29]. Studies were thus aimed to verify the likely involvement of NF-κB in redox changes consequent to GSH catabolism. Using murine V79-GGT cells, highly expressing a human GGT transgene, it was indeed shown that GGT-dependent ROS production induces the NF-κB-binding and transactivation activities. This induction mimicked the one observed by H_2O_2 and was inhibited by catalase, suggesting the involvement of GSH/GGT-derived H_2O_2 in the NF-κB activation [30].

However, studies carried out in human tumor cells showed that GSH/GGT-dependent modulation of NF-κB activation status can be more complex that firstly appreciated. Stimulation or inhibition of GGT activity in human melanoma Me665/2/60 cells resulted in stimulation or inhibition of NF-κB nuclear translocation, respectively [22]. The increased nuclear translocation following stimulation of GGT activity by the substrates glutathione and gly-gly was, however, paradoxically accompanied by decreased NF-κB DNA binding and gene transactivation (Fig. 4). NF-κB DNA binding could be restored by treating cell lysates with the thiol-reducing agent dithiothreitol (not shown), indicating the involvement of critical thiol groups by GSH/GGT-generated prooxidants. These observations indicate that reactions ensuing from GSH catabolism—while facilitating mobilization of NF-κB from cytoplasm to the nucleus—can ultimately down-regulate NF-κB DNA-binding and transcriptional activity, thus likely representing a mechanism for preventing excess NF-κB activation in conditions of persistent oxidative stress.

Besides NF-κB, modulatory effects by prooxidants and/or antioxidants have also been reported for AP-1 [31].

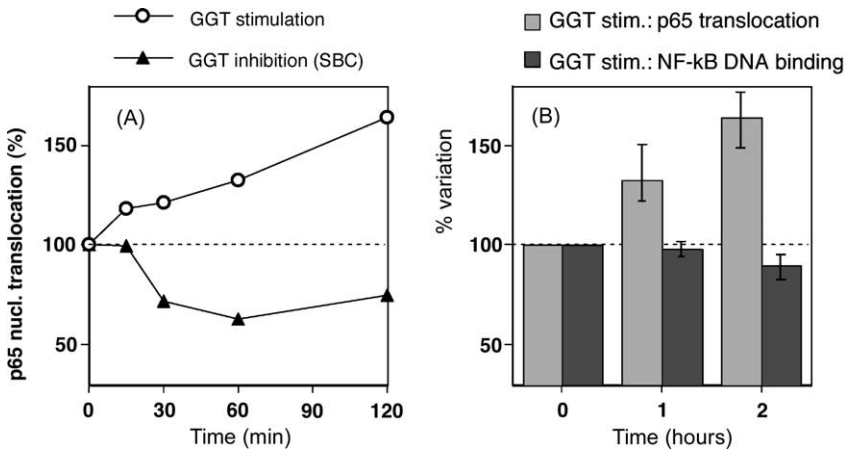

Fig. 4. Effects of GGT activity on p65 nuclear translocation (A and B) and NF-κB DNA binding (B) in human melanoma Me665/2/60 cells. Cells were incubated with GSH (200 μM) and gly-gly (2 mM) for GGT stimulation, in the presence or absence of the inhibitor SBC (10 mM). Translocation of p65 was determined in nuclear extracts, analyzed by western blot using an anti-p65 monoclonal antibody and ECL for revelation. DNA binding was determined by EMSA, using double-stranded biotin-labeled oligonucleotide probes and ECL for revelation. Quantification of ECL bands was obtained by densitometry. Mean values of two to four experiments are shown.

Interestingly, c-Jun DNA binding activity was recently shown to be redox-regulated through the reversible S-thiolation of a critical cysteine residue [32]; the same authors reported that the phenomenon could also involve the p50 component of NF-κB [33]. Studies in our laboratory have shown that the activation of GSH catabolism through GGT results in increased AP-1 DNA binding. Consistently, the GGT inhibitor acivicin suppressed this effect, confirming the role of GGT-mediated GSH catabolism. AP-1 DNA binding was suppressed also by the independent GGT inhibitor azaserine, as well as by catalase, Trolox C and deferoxamine, confirming that the effect is mediated through GGT-dependent, iron-catalyzed, oxidative mechanisms.[1]

Parallel studies have been extended to verify the possibility to detect oxidizing effects of GGT activity in specific proteins of the cell surface. The GGT-dependent oxidation and/or S-thiolation of accessible –SH groups of cell surface proteins might easily involve the extracellular domains of growth factor receptors, and this might induce conformational changes in their structure, thus potentially affecting their function. Several studies have documented that oxidative alteration of cysteines in receptor proteins can modify their ligand binding affinity and activation status [34–36]. It is therefore likely that the continuous flow of GGT-derived prooxidants can modulate the function of important growth factor receptors, as well as of receptors for apoptogenic ligands. To date we have investigated this aspect with the tumor necrosis factor receptor protein-1 (TNFR1), which is expressed at significant levels in melanoma Me665/2/60 cells. In presence of stimulation or inhibition of GGT activity, cells were reacted with cell-impermeant, thiol-specific biotinylated probes. After immuno-precipitation from cell lysates, TNFR1 protein was separated by SDS–PAGE and blotted. On blots TNFR1 protein was identified by means of antibodies, while its thiol redox status was revealed by means of streptavidin and enhanced chemiluminescence. Preliminary results indicate that the inhibition of GGT activity by two independent specific inhibitors (acivicin, serine/boric acid complex) indeed results in a marked increase of available –SH groups in TNFR1 protein (see footnote 1).

Modulating effects of GSH catabolism can be documented at further levels, on further molecular targets. A growing body of literature suggests that redox processes can affect both protein kinase and protein phosphatase (PP) activities [24,37]. Recent work on PPs has provided insight into how these enzymes might ‘‘sense’’ and transduce redox or oxidative stress conditions. All tyrosine PPs have a common active site, and a catalytic mechanism that involves the formation of a transient phosphoenzyme intermediate to the conserved cysteine residue [38]. The latter likely provides the molecular basis for the inactivating effect produced on PPs by hydrogen peroxide and by thiol-specific reagents. Modulating effect of redox reactions are also known for serine–threonine PPs [37]. Thus, the possibility exists that GGT-mediated prooxidant reactions may play a modulatory role of the cell ‘‘PPase tone.’’ In tumor cells expressing high GGT levels, this could be a mechanism by which transduction of signals through the kinase cascades is altered, with impairment of the cell responses to appropriate growth or death signals. A first set of experiments has been carried out to verify whether GGT-dependent prooxidant reactions could affect protein phosphorylation in Me665/2/60 melanoma cells. Preliminary results showed an increase of total phosphatase activity after hydrogen peroxide and stimulation of GGT activity. This phenomenon probably depended entirely on tyrosine-phosphatases, as in fact their activity was increased by

[1] Dominici *et al.*, manuscript in preparation.

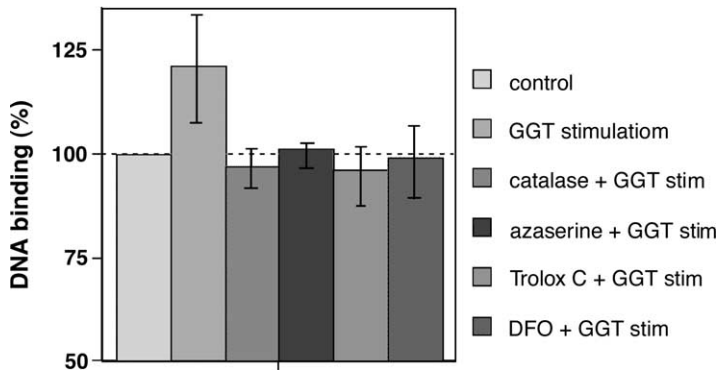

Fig. 5. Effects of GGT stimulation vs. inhibition on DNA binding of AP-1 in human melanoma Me665/2/60 cells. DNA binding was determined by EMSA, using double-stranded biotin-labeled oligonucleotide probes and ECL for revelation. Mean values of three to five experiments are shown.

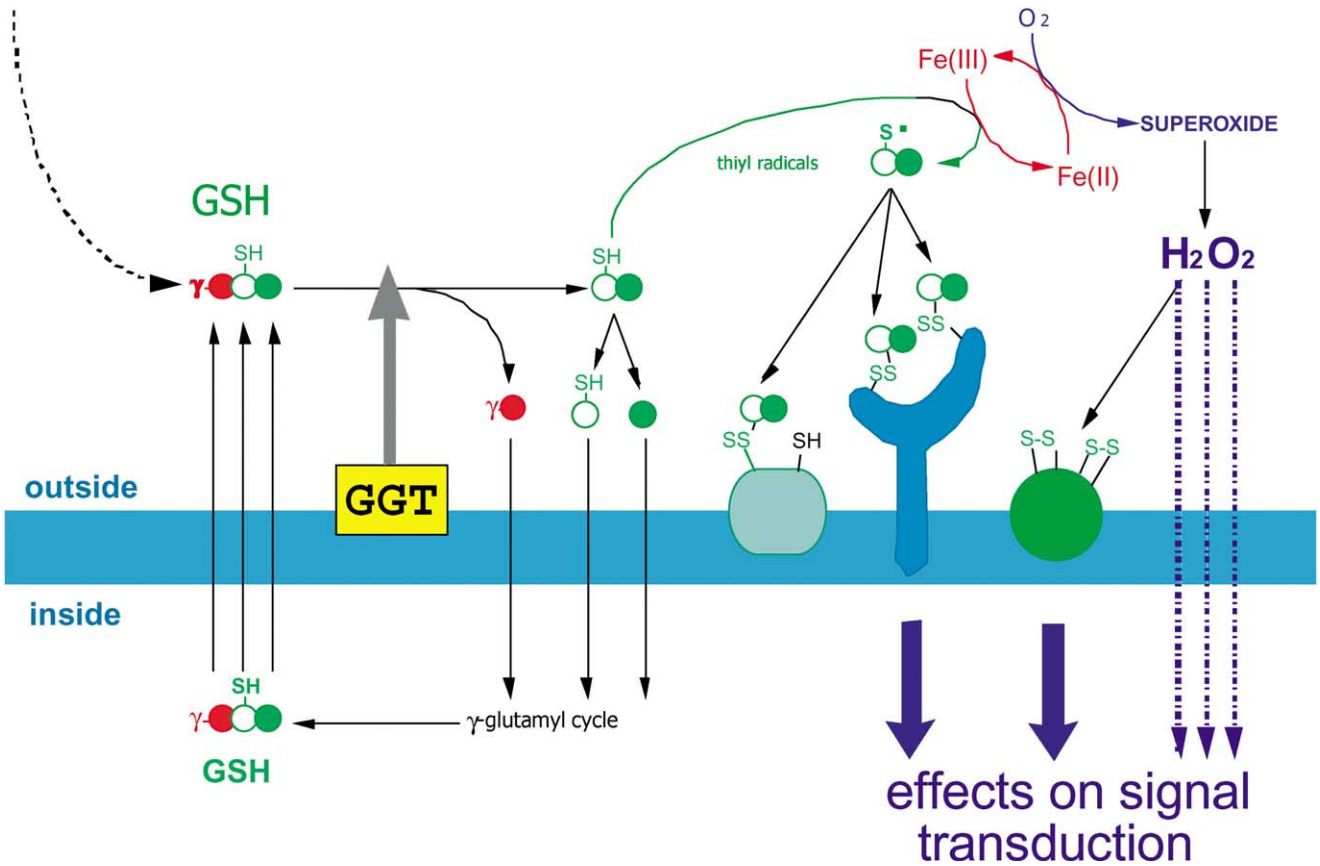

Fig. 6. Outline of the prooxidant reactions originating from GGT-dependent catabolism of extracellular GSH and of the potential molecular targets involved.

treatment with hydrogen peroxide—while, consistently, protein phosphotyrosine levels were decreased. Conversely, serine/treonine-phosphatase activities were decreased after both hydrogen peroxide and GGT stimulation. As observed in the case of NF-κB activation status, GGT activity and hydrogen peroxide seem to exert their action through the oxidation of critical thiols, since treatment with dithiothreitol could reverse these effects.[2]

[2] Pieri *et al.*, manuscript in preparation.

5. Concluding remarks

The experimental evidence obtained in our and other laboratories allows to describe a novel aspect of glutathione metabolism, i.e. the redox changes consequent to its cleavage by GGT. With the mediation of iron—and conceivably of other transition metals as well—GSH catabolism leads to the generation of ROS and thiyl radicals, whose prooxidant action is detectable on protein thiol groups in the first place (Figs. 5 and 6). Such processes

appear to involve a variety of cellular targets, including important elements of the signal trasduction chains. As GSH/GGT-dependent prooxidant reactions have been described by now in several distinct conditions and experimental models, these phenomena truly appear to represent a general pathophysiological process, with possible bearings on the understanding and treatment of several human disease conditions.

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