

Forum Original Research Communication

The S-Thiolating Activity of Membrane γ -Glutamyltransferase: Formation of Cysteinyl-Glycine Mixed Disulfides with Cellular Proteins and in the Cell Microenvironment*

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ABSTRACT

Previous studies have documented that activity of the plasma membrane enzyme γ -glutamyltransferase (GGT) is accompanied by prooxidant processes, with production of reactive oxygen species and oxidation of cellular protein thiols. The present work was aimed to verify the occurrence and extent of S-thiolation mediated by GGT and characterize the molecular species involved in mixed disulfide formation. Experiments show that the cysteinyl-glycine (CG) originating from cellular GGT-mediated glutathione (GSH) metabolism can efficiently thiolate cellular proteins, as well as proteins present in the extracellular environment. With cells presenting high levels of GGT expression, basal levels of CG-containing protein mixed disulfides are detectable, in cellular proteins, as well as in proteins of the culture medium. Stimulation of GGT activity in these cells by administration of substrates results in an increase of CG mixed disulfide formation and a concomitant decrease of GSH-containing disulfides, likely due to GGT-dependent removal of GSH from the system. The findings reported suggest that binding of CG ("protein S-cysteylglycylation") may represent an as yet unrecognized function of membrane GGT, likely playing a regulatory role(s) in the cell and its surroundings. *Antioxid. Redox Signal.* 7, 911–918.

INTRODUCTION

DESPITE ITS EARLY RECOGNIZED IMPORTANCE as a mechanism for regulation of protein function, protein S-thiolation has received in time less attention as compared with other well established modes of posttranslational protein modification, such as protein phosphorylation or glycosylation. This has been likely due to the scarcity of experimental evidence in support of the relevance *in vivo* of such protein modification. Most of the early studies were, in fact, mainly concerned with S-thiolation resulting from unphysiological oxidative stress conditions, in which the process appeared to proceed with little or no specificity as far as the proteins involved. The current interest in protein S-thiolation lies instead in its regulatory implications, deriving from the involvement in the process of specific target proteins, with definite effects on definite cellular functions. The fact that cys-

teine residues can undergo a range of graded modifications, each possibly corresponding to distinct functional states of a protein involved, *e.g.*, in signal transduction, has warranted them the recent definition as "nanotransducers in redox signaling" (3). Efforts have been dedicated in recent years to identify individual proteins susceptible to undergo S-thiolation, using, *e.g.*, thiol-specific cysteine-based probes (8). Regulation by S-thiolation has thus been reported for several important cell functions, including, *e.g.*, the activity of protein tyrosine phosphatases, the ubiquitin/proteasome system, the c-Jun DNA-binding activity, and many more (for review, see 12).

A few biochemical aspects of the S-thiolation process are, however, still in need of better elucidation. For example, it has been reported that in up to 85% of cases glutathione (GSH) is the species responsible for protein S-thiolation (1, 23). Other thiols also can form mixed disulfides with pro-

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*This paper is dedicated to Prof. Mario Comporti (Siena) on the occasion of his 70th birthday.

teins, producing *S*-cysteinylation (cysteine), *S*-homocysteinylation (homocysteine), and *S*-cysteamylation (cystamine) (29); nevertheless, the processes leading to these alternative *S*-thiolation modes have received little or no attention to date. In addition, although enzymatic activities capable of affecting dethiolation of proteins have been the subject of extensive studies, documenting the implication of thioredoxin, glutaredoxin, protein disulfide isomerase, and, more recently, phosphoglycerate kinase (14), much less is known about the endogenous (enzymatic) mechanisms in charge of promoting *S*-thiolation, with a possible regulatory significance in a more or less physiological context. Studies in this field are actually limited to the *S*-thiolating effects of phagocytic respiratory burst (22, 24).

We have previously shown that protein *S*-thiolation is one of several prooxidant effects ensuing from activation of the plasma membrane γ -glutamyltransferase (GGT), the ectoenzyme in charge of metabolizing extracellular glutathione. GGT activity, in fact, originates the dipeptide cysteinylglycine (CG), whose reactivity is responsible for reduction of metal cations and production of reactive oxygen species. In GGT-expressing cells, it was observed that these reactions are accompanied by oxidation of protein thiols, in the first place at the cell surface level (7). Against this background, the present work was aimed to verify if GGT-induced *S*-thiolation implies the binding to cellular proteins of CG itself, and if such process can involve proteins present in the extracellular environment, which could identify GGT as an intrinsic, physiologic *S*-thiolating activity of the cell.

MATERIALS AND METHODS

Chemicals

Unless otherwise indicated, all reagents, including cell culture media, were from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Cell transfection, culture, and treatments

Human metastatic melanoma Me665/2 cells were kindly provided by Dr. F. Zunino (Istituto Nazionale Tumori, Milan, Italy). The 2/21 clone, expressing only traces of GGT activity (0.2–0.7 mU/mg of protein), was used for the present study. In these cells, GGT was increased by stable transfection with a vector (pcDNA; Invitrogen) containing GGT cDNA. A lipid transfection according to Lipotaxi[®] mammalian transfection kit protocol (Stratagene) was used. Of the several subclones thus generated, two were chosen: 2/21/11 cells ("clone 11"), in which no increase of GGT activity was induced, were used as GGT-negative controls. For comparison, 2/21/7 cells ("clone 7") were chosen, presenting a high GGT activity after transfection (70–75 mU/mg of protein).

Cells were cultured in RPMI 1640 medium, supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 2 mM L-glutamine, and 0.5 mg/ml Geneticin (G418, Invitrogen), at 37°C, in a 5%/95% CO₂/air atmosphere. Incubations were carried out in Hanks' balanced salt solution (HBSS) medium. For stimulation of GGT activity, the substrates GSH and glycylglycine (Gly2)—the latter serving as acceptor for the γ -

glutamyl moiety (11)—were added to incubations at a final concentration of 2 and 20 mM, respectively. Where indicated, human apo-transferrin (Apo-Tf) was also added to the incubation medium (0.3 mg/ml). Inhibition of GGT activity was obtained by treating cells with noncompetitive irreversible inhibitor acivicin (AT125; 1 mM final concentration) for 60 min prior to incubation. All components were freshly prepared and kept on ice until use.

Sample preparation, protein reduction, and fluorescent derivatization of thiols

For determination of free thiols, aliquots of incubation mixtures were diluted in 0.2 M Tris-HCl buffer, pH 8.2, containing 20 mM EDTA and 1% (wt/vol) sodium dodecyl sulfate (SDS). For measurements on cellular proteins, cells were washed twice with phosphate-buffered saline and treated with 10% trichloroacetic acid (TCA; 20 min, 4°C). For measurements on serum proteins of culture media, aliquots were acidified with 10% TCA (Fluka) and centrifuged at 15,000 g (10 min at 4°C). In all cases, protein precipitates were washed twice with diethyl ether and resuspended in 0.2 M Tris-HCl buffer, pH 8.2, containing 20 mM EDTA and 1% (wt/vol) SDS. Samples and standards were incubated (30 min, room temperature) with tris(2-carboxyethyl)phosphine (Molecular Probes, Eugene, OR, U.S.A.), 5 g/L final concentration, to achieve disulfide reduction. Proteins were then precipitated with 5% TCA (final concentration); samples were vortex-mixed immediately and centrifuged (10 min, 15,000 g).

After centrifugation, aliquots (100 μ l) of the supernatants were added to test tubes prepared with 250 μ l of 125 mM borate buffer, pH 9.5, containing 4 mM EDTA, 110 μ l of a 1 g/l solution of 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F; Fluka), and 20 μ l of 1.55 M NaOH. Derivatization was carried out for 60 min, at 60°C in the dark. After derivatization, samples were acidified with 20 μ l of 0.4 M H₃PO₄ and kept at –20°C until HPLC analysis.

HPLC measurements

Determination of low-molecular-weight thiols was carried out as described (9, 21). Reverse-phase HPLC was performed using a Resolve[®] C18 column (Waters, U.S.A.), and a Beckman 125P pump equipped with a Rheodyne 7125 injection valve, fitted with a 200- μ l sample loop. Mobile phase (5% methanol in 0.2 M KH₂PO₄, adjusted to pH 2.7 with H₃PO₄; filtered and degassed before use) was delivered at a flow rate of 0.6 ml/min. Calibration was based on external standards using stock solutions (10 mM) of Cys, CG, and GSH prepared in 0.12 M perchloric acid and stored at –20°C; working solutions were prepared daily in 0.2 M Tris-HCl, pH 8.2, containing 20 mM EDTA and 1% (wt/vol) SDS. A Shimadzu RF-551 fluorimetric detection system was used (filter settings: 385 nm excitation, 515 nm emission).

Other determinations

Protein content was determined by the method of Bradford (Bio-Rad Protein Assay). GGT activity was determined spectrophotometrically using γ -glutamyl-*p*-nitroanilide as substrate, according to Huseby and Strömme (11) with minor

modifications. Statistical analysis of data was performed by ANOVA.

RESULTS

A time course of GSH consumption by purified GGT in a chemical, acellular system, with concomitant generation of equimolar concentrations of CG, is shown in Fig. 1A and B. For evaluation of GSH metabolism affected by membrane-bound GGT activity, substrates were added to melanoma cells and all determinations were made at 30 min of incubation. As reported in Fig. 1C, a decrease of GSH levels occurred in the medium of GGT-negative (control) clone 11 cells, an effect

likely due to GSH spontaneous oxidation; such decrease was not prevented by GGT inhibition. On the other hand, the GSH consumption by clone 7 cells (Fig. 1C) was accompanied by a concomitant production of equimolar CG (Fig. 1D), and pretreatment of clone 7 cells with the GGT inhibitor AT125 suppressed the phenomenon.

The extent of protein S-thiolation induced during GGT-mediated GSH metabolism was evaluated after addition of the acceptor substrate Gly2 (= activation of GGT) to incubations already containing GSH (Fig. 2). No significant differences were present before and after activation of GGT, in neither clone 11 nor clone 7 cells (Fig. 2A). On the other hand, activation of GGT in clone 7 cells resulted in a marked increase of CG bound to cellular protein, and the effect was largely

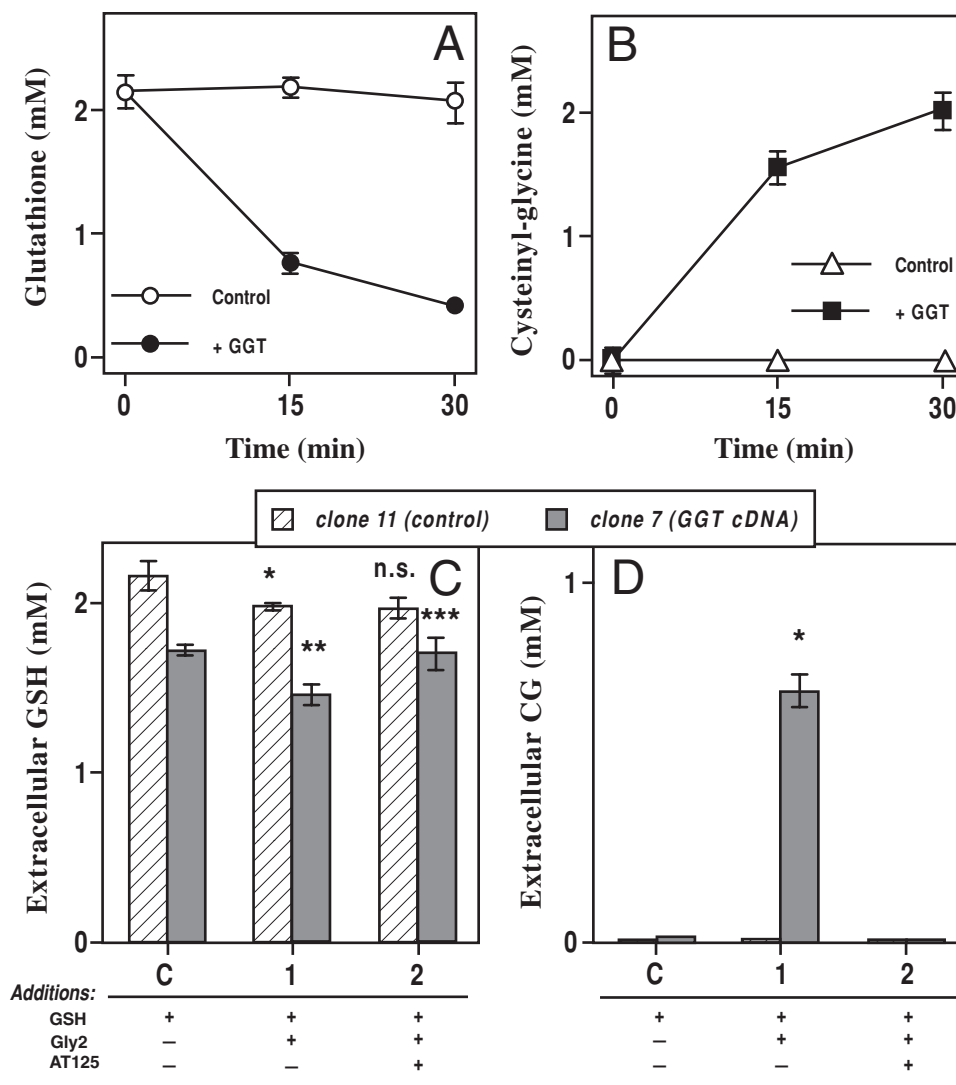


FIG. 1. GSH consumption with production of CG by purified and cell membrane-bound GGT activity. (A and B) Experiments with purified GGT. Incubations were carried out at 37°C in 100 mM Tris-HCl, pH 7.4, and included GSH (2 mM), Gly2 (20 mM), and GGT (200 mU/ml), as indicated. (C and D) Experiments with melanoma cells. Incubations were carried out at 37°C in HBSS medium, pH 7.4, and included GSH (2 mM), Gly2 (20 mM), and AT125 (1 mM), as indicated. Results are means \pm SD of three separate experiments. * p < 0.02 as compared with corresponding sample C; ** p < 0.005 as compared with corresponding sample C; *** p < 0.001 as compared with corresponding sample 1; n.s., not significantly different from corresponding sample 1.

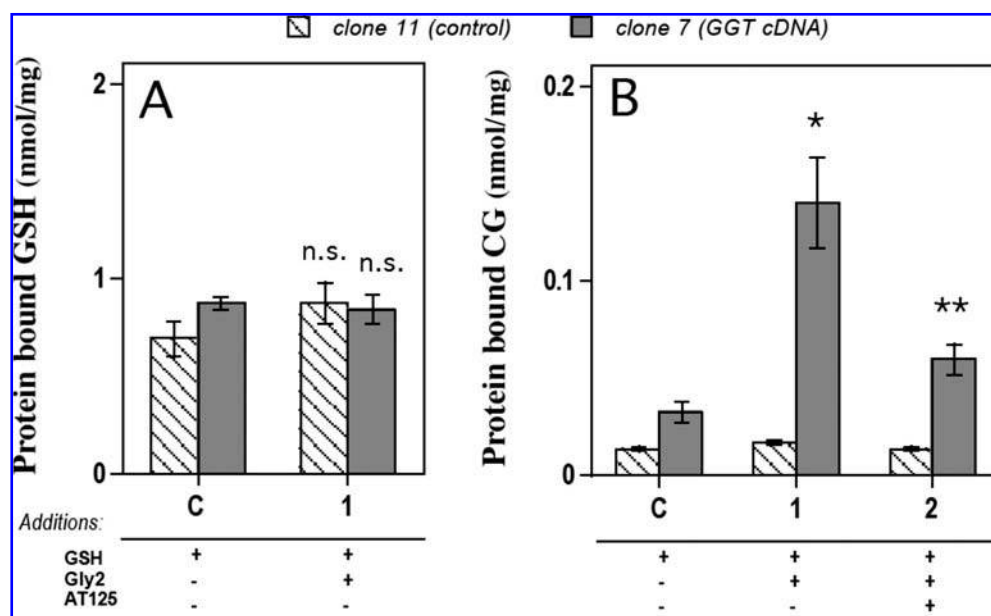


FIG. 2. S-Thiolation of cellular proteins induced after activation of membrane GGT melanoma cell clones. (A) Protein-bound GSH. (B) Protein-bound CG. Determinations were made after 30 min of incubation of cells in HBSS, to which GSH (2 mM), Gly2 (20 mM), and AT125 (1 mM) were added, as indicated. Results are means \pm SD of three separate experiments. * p < 0.001 as compared with corresponding sample C; ** p < 0.005 as compared with sample 1; n.s., difference not significant as compared with corresponding sample C.

prevented by AT125 (Fig. 2B). Protein-bound CG levels were much lower than those of protein-bound GSH, reaching a maximum of approximately 1/10 in GGT-stimulated cells.

The possibility that basal (unstimulated) GGT activity might induce differences in the mode and extent of basal S-thiolation of cellular proteins was verified in experiments reported in Fig. 3. Resting, untreated GGT-expressing clone 7 cells indeed presented a significantly lower extent of S-glutathionylation (approximately -35%), as well as a significantly higher CG binding to protein (approximately +70%).

Again, protein-bound CG was much lower than GSH (approximately 1/20 in GGT-expressing cells).

A model exogenous protein, purified human Apo-Tf, was used in experiments aimed to verify whether GGT-deriving CG might affect S-thiolation of proteins in the extracellular medium. GGT activation in clone 7 cells caused a decrease in the extent of GSH bound to Apo-Tf (approximately -25%, Fig. 4A), whereas—although at lower concentrations—binding of CG was remarkably increased (Fig. 4B). Binding of CG to extracellular proteins was also detected in basal culture

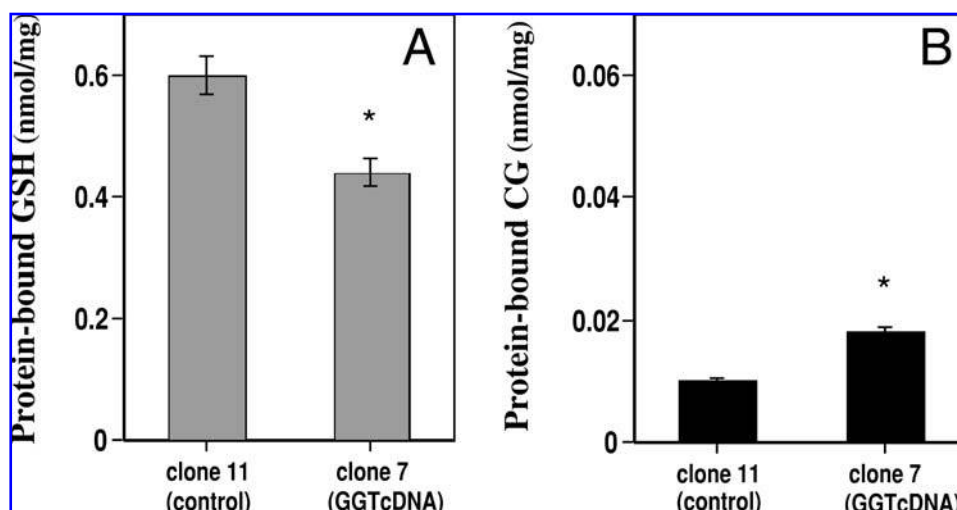


FIG. 3. Basal levels of protein S-thiolation in melanoma cell clones. (A) Protein-bound GSH. (B) Protein-bound CG. Cells were cultured in RPMI 1640 medium, and determinations were performed 48 h after change of medium. No additions were made during the incubation. Results are means \pm SD of three separate experiments. * p < 0.001.

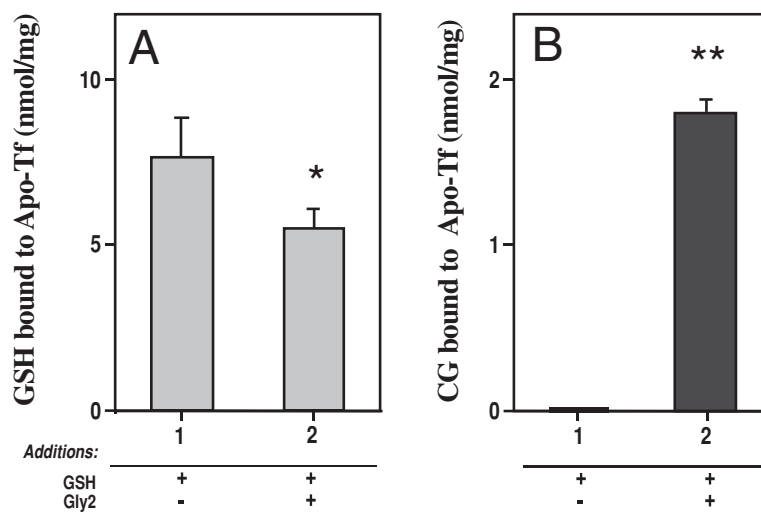


FIG. 4. *S*-Thiolation of purified human Apo-Tf added as target protein in the incubation medium of clone 7 (GGT-expressing) melanoma cells: effects of GGT activation. (A) Protein-bound GSH. (B) Protein-bound CG. Determinations were made after 30 min of incubation of cells in HBSS medium, to which Apo-Tf had been added at 0.3 mg/ml final concentration. Experiments were started by addition of GSH and Gly2, at 2 and 20 mM, final concentration. Results are means \pm SD of three separate experiments. * $p < 0.05$; ** $p < 0.001$.

conditions, to proteins contained in routine culture medium (Fig. 5). After 48 h of culture, both cell clones studied had produced changes in *S*-thiolation of culture medium proteins: with GGT-expressing clone 7 cells, levels of protein-bound CG were considerably increased, whereas protein-bound GSH was decreased. The reverse was true with GGT-negative clone 11 cells, which increased *S*-glutathionylation of proteins in the culture medium, while decreasing protein-bound CG.

DISCUSSION

Membrane GGT expression and activity should be included among the factors affecting cellular redox-sensitive functions, through effects on redox status of protein thiols.

Previous work from our laboratory has, in fact, documented that GGT enzyme activity can initiate prooxidant reactions in the cell microenvironment, and that this is accompanied by reversible oxidation of thiols in cell proteins (5, 15), including receptors of the cell surface (7). Protein *S*-thiolation is one of the mechanisms of such protein thiol oxidation (5). The prooxidant effects of GGT activity were shown to be mediated by CG, the major metabolite of GSH. Due to its lower pK_a , CG can easily interact with metal cations, thus promoting redox cycling phenomena (17, 24), eventually resulting in production of reactive oxygen species (5) and stimulation of oxidative processes, *e.g.*, lipid peroxidation (16, 26). The occurrence of the described GGT-induced prooxidant reactions was shown by us in a series of pathophysiologic conditions (for review, see 6), and their effects have been detected at several levels in the cellular machinery (for review, see 18).

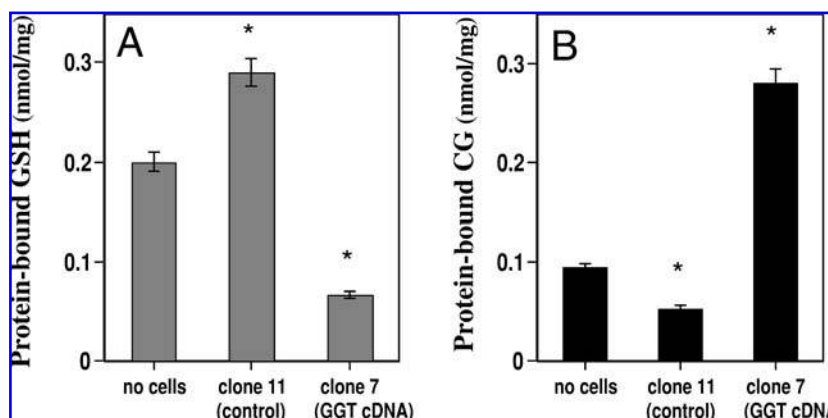


FIG. 5. Basal levels of *S*-thiolation in proteins of the culture medium of melanoma cell clones. (A) Protein-bound GSH. (B) Protein-bound CG. Cells were cultured in RPMI, and determinations were performed 48 h after change of medium. Where indicated, culture medium was incubated in the absence of cells. No additions were made during the 48 h of incubation. Results are means \pm SD of three separate experiments. * $p < 0.001$.

Data reported in the present study show that CG is produced extracellularly during the GSH catabolism mediated by cell membrane GGT, and is capable of affecting *S*-thiolation of cellular as well as extracellular proteins. CG-containing mixed disulfides were formed as a result of activation of GGT, obtained by administration to cells of its substrates GSH and γ -glutamyl acceptor Gly2, but are also detectable in resting, untreated GGT-expressing cells, suggesting that formation of CG-containing mixed disulfides is occurring in basal conditions, during routine GGT activity.

In all instances, the amount of CG recovered after reduction of cellular or extracellular protein was five- to 10-fold lower than the concomitant GSH recovery. In fact, extracellular CG is normally hydrolyzed by membrane dipeptidases to cysteine and glycine (10); only a minor part of the CG produced may be thus available long enough as to make redox reactions with protein. It should be considered, however, that the measurements were performed on total, unfractionated proteins, whereas binding of CG might be concentrated on specific individual proteins—cellular and/or extracellular. In the case of *S*-glutathionylation, the occurrence in protein structure of defined amino acid sequences (sort of a “GSH footprint”) (12), capable of establishing suitable electrostatic interactions with negatively charged carboxyl groups in the GSH molecule, has been invoked to explain the susceptibility of specific proteins to bind GSH at specific sites. With respect to CG binding, it cannot be excluded that akin “CG footprints” may exist in the structure of specific proteins. An additional factor possibly addressing the binding of CG toward specific proteins lies in the location occupied by GGT itself, *i.e.*, the outer plasma membrane surface, which might result in a concentration of CG binding on proteins of the plasma membrane and the extracellular microenvironment. Previous studies documented that GGT-dependent prooxidant effects are occurring at the plasma membrane level and extracellularly (5, 15). The modulating effects of CG-*S*-thiolation could be then concentrated on proteins in the plasma membrane (*e.g.*, receptors, transporters) and its vicinity (*e.g.*, proteins of the basement membrane, in the case of epithelial cells).

In some experiments, CG binding to protein was accompanied by a significant decrease in *S*-glutathionylation (Figs. 3 and 4). This can be explained by the fact that the presence of active GGT on cells acts as a sort of GSH sink, removing it from the system (compare Fig. 1). The same explanation can be given for the higher extracellular *S*-glutathionylation observed with (GGT-negative) clone 11 cells in basal conditions (Figs. 3A and 5A). The decreased *S*-glutathionylation apparent in Figs. 3 and 4 does not correspond, however, to a shift of proteins toward a more reduced state; in fact, GGT activation was previously shown to induce a marked net decrease in reduced SH groups of proteins (5, 15). Rather, it is conceivable that the release of GSH from protein may be part of an overall oxidative rearrangement of protein thiols induced after the prooxidant processes ensuing from GGT activation. Such rearrangement appears to include (a) the appearance of CG-containing mixed disulfides, (b) an increased oxidation of protein SH groups of some other nature (allegedly production of sulfenic acids and related

species), and (c) a decreased protein *S*-glutathionylation, likely as a result of a and b.

The significance of GGT-dependent *S*-thiolation can be twofold. Firstly, protein *S*-thiolation has been interpreted as a defense against irreversible oxidative damage (2, 24, 29). In this perspective, as GGT is often expressed at high levels in primitive as well as metastatic tumors, this aspect of its function might well concur in the resistance of cancer cells against cytotoxic effects of oxidative stress, as in the case of several important prooxidant anticancer drugs. We have indeed described a role of GGT in cell resistance to cytotoxicity of platinum drugs (4, 19, 20). Secondly, a more intriguing possibility is that the GGT-induced formation of CG-containing mixed disulfides—a process that, by analogy, can be termed “*S*-cysteylglycylation”—may have a regulatory function, similar to the case of *S*-glutathionylation. Further studies are required to elucidate this point. In the meantime, the fact that in some experiments *S*-cysteylglycylation was consistently accompanied by a decrease in the extent of *S*-glutathionylation can already be considered as an (indirect) mode of thiol-based regulation of sensitive protein targets.

Perspectives

Our results allow the characterization of *S*-thiolation as a major function of membrane GGT enzyme activity. The binding of CG to proteins may represent a molecular variant of *S*-thiolation implying specific regulatory effects, *i.e.*, possibly different from those mediated by *S*-glutathionylation. GGT-dependent protein *S*-cysteylglycylation may possess some degree of target specificity in that GGT prooxidant reactions are primarily directed outwards, so that its effects on proteins of the cell surface and of the cell microenvironment may be predominant. A process of this kind is likely to induce redox modifications of critical thiols in receptor proteins and downstream signal transduction. Moreover, the observed GGT-induced *S*-thiolation of extracellular proteins raises the possibility that this process may represent a tool by which GGT-expressing cells—notably a large variety of cancer cells (27)—can modulate redox status and function of important proteins in the extracellular matrix and on the surface of other by-standing cells, *e.g.*, immune cells or endothelium.

ACKNOWLEDGMENTS

This work was supported by Associazione Italiana Ricerca sul Cancro (A.I.R.C., Italy) and the Italian Ministry for Education, University and Scientific Research (MIUR-FIRB 2001 Funds).

ABBREVIATIONS

Apo-Tf, apo-transferrin; AT125, acivicin; CG, cysteinylglycine; GGT, γ -glutamyltransferase; Gly2, glycyl-glycine; GSH, glutathione; HBSS, Hanks' balanced salt solution; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid.

REFERENCES

1. Chai YC, Hendrich S, and Thomas JA. Protein S-thiolation in hepatocytes stimulated by *t*-butyl hydroperoxide, menadione and neutrophils. *Arch Biochem Biophys* 310: 264–272, 1994.
2. Coan C, Ji JY, Hideg K, and Mehlhorn RJ. Protein sulfhydryls are protected from irreversible oxidation by conversion to mixed disulfides. *Arch Biochem Biophys* 295: 369–378, 1992.
3. Cooper CE, Patel RP, Brookes PS, and Darley-Usmar VM. Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem Sci* 27: 489–492, 2002.
4. Daubeuf S, Leroy P, Paolicchi A, Pompella A, Wellman M, Galteau MM, and Visvikis A. Enhanced resistance of HeLa cells to cisplatin by overexpression of gamma-glutamyltransferase. *Biochem Pharmacol* 64: 207–216, 2002.
5. Dominici S, Valentini M, Maellaro E, Del Bello B, Paolicchi A, Lorenzini E, Tongiani R, Comporti M, and Pompella A. Redox modulation of cell surface protein thiols in U937 lymphoma cells: the role of γ -glutamyl transpeptidase-dependent H_2O_2 production and S-thiolation. *Free Radic Biol Med* 27: 623–635, 1999.
6. Dominici S, Paolicchi A, Lorenzini E, Maellaro E, Comporti M, Pieri L, Minotti G, and Pompella A. Gamma-glutamyltransferase-dependent prooxidant reactions: a factor in multiple processes. *Biofactors* 17: 187–198, 2003.
7. Dominici S, Pieri L, Paolicchi A, De Tata V, Zunino F, and Pompella A. Endogenous oxidative stress induces distinct redox forms of tumor necrosis factor receptor-1 in melanoma cells. *Ann NY Acad Sci* 1030: 62–68, 2004.
8. Eaton P, Byers HL, Leeds N, Ward MA, and Shattock MJ. Detection, quantitation, purification and identification of cardiac proteins S-thiolated during ischemia and reperfusion. *J Biol Chem* 277: 9806–9811, 2002.
9. Frick B, Schrocksnadel K, Neurauter G, Wirleitner B, Artner-Dworzak E, and Fuchs D. Rapid measurement of total plasma homocysteine by HPLC. *Clin Chim Acta* 331: 19–23, 2003.
10. Griffith OW and Meister A. Glutathione: interorgan translocation, turnover, and metabolism. *Proc Natl Acad Sci USA* 76: 5606–5610, 1979.
11. Huseby NE and Strömme JH. Practical points regarding routine determination of γ -glutamyl transferase (γ -GT) in serum with a kinetic method at 37°C. *Scand J Clin Lab Invest* 34: 357–361, 1974.
12. Klatt P and Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. *Eur J Biochem* 267: 4928–4944, 2000.
13. Klatt P, Molina EP, De Lacoba MG, Padilla CA, Martinez-Galesto E, Barcena JA, and Lamas S. Redox regulation of c-Jun DNA binding by reversible S-glutathiolation. *FASEB J* 13: 1481–1490, 1999.
14. Lay AJ, Jiang XM, Kisker O, Flynn E, Underwood A, Condrón R, and Hogg PJ. Phosphoglycerate kinase acts in tumor angiogenesis as a disulfide reductase. *Nature* 408: 869–873, 2000.
15. Maellaro E, Dominici S, Del Bello B, Valentini MA, Pieri L, Perego P, Supino R, Zunino F, Lorenzini E, Paolicchi A, Comporti M, and Pompella A. Membrane gamma-glutamyl transpeptidase activity of melanoma cells: effects on cellular H_2O_2 production, cell surface protein thiol oxidation and NF- κ B activation status. *J Cell Sci* 113: 2671–2678, 2000.
16. Paolicchi A, Tongiani R, Tonarelli P, Comporti M, and Pompella A. Gamma-glutamyl transpeptidase-dependent lipid peroxidation in isolated hepatocytes and HepG2 hepatoma cells. *Free Radic Biol Med* 22: 853–860, 1997.
17. Paolicchi A, Minotti G, Tonarelli P, Tongiani R, De Cesare D, Mezzetti A, Dominici S, Comporti M, and Pompella A. Gamma-glutamyl transpeptidase-dependent iron reduction and low density lipoprotein oxidation—a potential mechanism in atherosclerosis. *J Invest Med* 47: 151–160, 1999.
18. Paolicchi A, Dominici S, Pieri L, Maellaro E, and Pompella A. Glutathione catabolism as a signalling mechanism. *Biochem Pharmacol* 64: 1029–1037, 2002.
19. Paolicchi A, Lorenzini E, Perego P, Supino R, Zunino F, Comporti M, and Pompella A. Extracellular thiol metabolism in clones of human metastatic melanoma with different gamma-glutamyl transpeptidase expression—implications for cell response to platinum-based drugs. *Int J Cancer* 97: 740–745, 2002.
20. Paolicchi A, Sotiropoulou M, Perego P, Daubeuf S, Visvikis A, Lorenzini E, Franzini M, Romiti N, Chieli E, Leone R, Apostoli P, Colangelo D, Zunino F, and Pompella A. Gamma-glutamyl transpeptidase catalyzes the extracellular detoxification of cisplatin in a human cell line derived from the proximal convolute tubule of the kidney. *Eur J Cancer* 39: 996–1003, 2003.
21. Pfeiffer CM, Huff DL, and Gunter EW. Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. *Clin Chem* 45: 290–292, 1999.
22. Ravichandran V, Seres T, Moriguchi T, Thomas JA, and Johnston RB Jr. S-Thiolation of glyceraldehyde-3-phosphate dehydrogenase induced by the phagocytosis-associated respiratory burst in blood monocytes. *J Biol Chem* 269: 25010–25015, 1994.
23. Schuppe-Koistinen I, Gerdes R, Moldéus P, and Cotgreave IA. Studies on the reversibility of protein S-thiolation in human endothelial cells. *Arch Biochem Biophys* 315: 226–234, 1994.
24. Seres T, Ravichandran V, Moriguchi T, Rokutan K, Thomas JA, and Johnston RB Jr. Protein S-thiolation and dethiolation during the respiratory burst in human monocytes—a reversible post-translational modification with potential for buffering the effects of oxidant stress. *J Immunol* 156: 1973–1980, 1996.
25. Spear N and Aust SD. Thiol-mediated NTA-Fe(III) reduction and lipid peroxidation. *Arch Biochem Biophys* 312: 198–202, 1994.
26. Stark A-A, Zeiger E, and Pagano DA. Glutathione metabolism by γ -glutamyltranspeptidase leads to lipid peroxidation: characterization of the system and relevance to hepatocarcinogenesis. *Carcinogenesis* 14: 183–189, 1993.

27. Tew KD, Monks A, Barone L, Rosser D, Akerman G, Montali JA, Wheatley JB, and Schmidt DE Jr. Glutathione-associated enzymes in the human cell lines of the National Cancer Institute Drug Screening Program. *Mol Pharmacol* 50: 149–159, 1996.
28. Thomas JA, Chai YC, and Jung CH. Protein S-thiolation and dethiolation. *Methods Enzymol* 233: 385–395, 1994.
29. Thomas JA, Poland B, and Honzatko R. Protein sulfhydryls and their role in the antioxidant function of protein S-thiolation. *Arch Biochem Biophys* 319: 1–9, 1995.

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Received for publication October 14, 2004; accepted January 19, 2005.

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2. Francesco Galli, Andrea Battistoni, Roberto Gambari, Alfonso Pompella, Alessandra Bragonzi, Francesca Pilolli, Luigi Iuliano, Marta Piroddi, Maria Cristina Dehecchi, Giulio Cabrini. 2011. Oxidative stress and antioxidant therapy in cystic fibrosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [[CrossRef](#)]
3. Ying Xiong , Joachim D. Uys , Kenneth D. Tew , Danyelle M. Townsend . 2011. S-Glutathionylation: From Molecular Mechanisms to Health Outcomes. *Antioxidants & Redox Signaling* **15**:1, 233-270. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
4. Alfonso Pompella, Michele Emdin, Maria Franzini, Aldo Paolicchi. 2009. Serum #-glutamyltransferase: linking together environmental pollution, redox equilibria and progression of atherosclerosis?. *Clinical Chemistry and Laboratory Medicine* **47**:12, 1583-1584. [[CrossRef](#)]
5. M. Ferretti, T. Destro, S. C. E. Tosatto, N. La Rocca, N. Rascio, A. Masi. 2009. Gamma-glutamyl transferase in the cell wall participates in extracellular glutathione salvage from the root apoplast. *New Phytologist* **181**:1, 115-126. [[CrossRef](#)]
6. Maria Franzini, Alessandro Corti, Barbara Martinelli, Antonella Del Corso, Michele Emdin, Giuliano F. Parenti, Mattia Glauber, Alfonso Pompella, Aldo Paolicchi. 2009. #-Glutamyltransferase activity in human atherosclerotic plaques—Biochemical similarities with the circulating enzyme. *Atherosclerosis* **202**:1, 119-127. [[CrossRef](#)]
7. Alessandro Corti, Maria Franzini, Alessandro F. Casini, Aldo Paolicchi, Alfonso Pompella. 2008. Vitamin C supply to bronchial epithelial cells linked to glutathione availability in elf — A role for secreted #-glutamyltransferase?. *Journal of Cystic Fibrosis* **7**:2, 174-178. [[CrossRef](#)]
8. A MASI, T DESTRO, L TURETTA, S VAROTTO, G CAPORALE, M FERRETTI. 2007. Localization of gamma-glutamyl transferase activity and protein in Zea mays organs and tissues. *Journal of Plant Physiology* **164**:11, 1527-1535. [[CrossRef](#)]
9. Michele Emdin, Claudio Passino, Maria Franzini, Aldo Paolicchi, Alfonso Pompella. 2007. #-glutamyltransferase and pathogenesis of cardiovascular diseases. *Future Cardiology* **3**:3, 263-270. [[CrossRef](#)]
10. Inga Kwiecie#, Hanna Rokita, El#bieta Lorenc-Koci, Maria Sokolowska, Lidia W#odek. 2007. The effect of modulation of ?-glutamyl transpeptidase and nitric oxide synthase activity on GSH homeostasis in HepG2 cells. *Fundamental & Clinical Pharmacology* **21**:1, 95-103. [[CrossRef](#)]
11. Maria Franzini, Alessandro Corti, Evelina Lorenzini, Aldo Paolicchi, Alfonso Pompella, Michelandrea De Cesare, Paola Perego, Laura Gatti, Roberto Leone, Pietro Apostoli, Franco Zunino. 2006. Modulation of cell growth and cisplatin sensitivity by membrane #-glutamyltransferase in melanoma cells. *European Journal of Cancer* **42**:15, 2623-2630. [[CrossRef](#)]
12. Suryakant K. Niture, Chinavenmeni S. Velu, Nathan I. Bailey, Kalkunte S. Srivenugopal. 2005. S-Thiolation mimicry: Quantitative and kinetic analysis of redox status of protein cysteines by glutathione-affinity chromatography. *Archives of Biochemistry and Biophysics* **444**:2, 174-184. [[CrossRef](#)]
13. Philip Eaton , Michael J. Shattock . 2005. Protein S-Thiolation: Emphasis on Cell Signaling and Gene Expression. *Antioxidants & Redox Signaling* **7**:7-8, 839-840. [[Citation](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]