IUBMB *Life*, 57(3): 189–192, March 2005

Taylor & Francis
Taylor & Francis Group

Is There an Answer?

Is There An Answer? is intended to serve as a forum in which readers to IUBMB Life may pose questions of the type that intrigue biochemists but for which there may be no obvious answer or one may be available but not widely known or easily accessible.

Readers are invited to e-mail f.vella@sasktel.net if they have questions to contribute or if they can provide answers to questions that are provided here from time to time. In the latter case, instructions will be sent to interested readers. Answers should be, whenever possible, evidence-based and provide relevant references. – Frank Vella

Q & A

Question: Is there a role for S-glutathionylation of proteins in human disease?

Answer: Isabella Dalle-Donne[1]*, Ranieri Rossi[2], Daniela Giustarini[2], Roberto Colombo[1], and Aldo Milzani[1]
[1]Department of Biology, University of Milan, via Celoria 26, I-20133, Milan, Italy;
[2]Department of Neuroscience, University of Siena, via A. Moro 4, I-53100, Siena, Italy;
***E-mail: quack@unimi.it**

Oxidative stress describes a condition in which cellular antioxidant defences are inadequate to completely detoxify the reactive oxygen/nitrogen species (RONS) generated, as a result of excessive production of RONS and/or loss of antioxidant defences, so that proteins, lipids, DNA, and other molecules undergo a number of oxidative modifications. Progressive accumulation of macromolecular oxidative damage can lead to loss or decrease of cellular and tissue functions or gain of harmful functions, and, ultimately, cell death. Oxidative stress occurs in many human diseases and may make significant contribution to their pathogenesis. Consequently, a battery of biomarkers of oxidative stress status in humans is available or under development (*1*).

Glutathione is 2–3 orders of magnitude more abundant than any other redox-active compound, such as cysteine, $\text{thioredoxin}_{red/ox}$, or $NAD(P)H/NAD(P)^+$, and is widely considered to be the major determinant and indicator of the overall/cellular redox state (*2*). In particular, the amounts and the ratio of reduced (GSH) and oxidized (GSSG) glutathione in blood are considered reliable indexes of whole-body GSH status and of overall redox status. A decrease in GSH and an increase in GSSG concentration, and changes in the GSH/GSSG ratio may be contributing factors in the pathogenesis of, for example, chronic obstructive pulmonary disease, acute respiratory distress syndrome, AIDS, pre-eclampsia, cardiovascular disease, diabetes mellitus, rheumatoid arthritis, and of amyotrophic lateral sclerosis, Parkinson disease (PD), and Alzheimer disease (AD) (*3*). Further, cataractous lenses show a decrease in GSH/GSSG ratio compared with clear lenses (*4*).

A significant amount of glutathione in cells may be reversibly bound to sulfhydryl groups of proteins (PSH) by *S*-glutathionylation to form *S*-glutathionylated proteins (PSSG). PSSG formation is an early cellular response to mild oxidative stress, but glutathionylation/deglutathionylation is a dynamic process that also occurs under basal physiological conditions (*4–6*), suggesting its possible involvement in signalling and redox regulation of protein functions (*2, 7*). *S*-Glutathionylation can occur by: (i) direct interaction between partially oxidised PSH (thiyl radical or sulfenic acid intermediates) and GSH; or (ii) thiol/disulphide exchange reactions between PSH and GSSG or PSSG (*2, 7*). Unless intracellular GSSG concentration reaches unusually high levels, GSSG is unlikely to mediate PSSG formation in cells (*8*). Glutathione-thiyl radical, *S*-nitrosoglutathione, and glutathione disulphide *S*-oxide are alternative mediators of PSSG formation (*9, 10*). PSSG can be reduced to PSH by glutaredoxin or by non-catalysed reaction with GSH, once the restoration of an appropriate GSH/GSSG ratio is underway.

PSSG have been investigated as possible biomarkers of oxidative stress in human disease (*1, 7*). *S*-Glutathionyl-haemoglobin concentration is increased in type I and type II diabetes, Friedreich's ataxia, hyperlipidemia, uremia associated with haemodialysis or peritoneal dialysis, and in smokers, and has been proposed as a useful biomarker of whole-body oxidative stress in humans (*7*). In Friedreich's ataxia, the most common of the hereditary ataxias, significant increase in glutathionyl-haemoglobin and glutathionyl-actin occurs in blood and fibroblasts, respectively (*6, 7*). *S*-Glutathionyl-actin has been identified in several cells during reperfusion of ischemic rat heart (*11*), in oxidatively-stressed human T cell blasts (*12*), and in human hepatoma

Received 4 February 2005; accepted 16 February 2005

ISSN 1521-6543 print/ISSN 1521-6551 online © 2005 IUBMB
DOI: 10.1080/15216540500090579

cells (*13*). In an *in vitro* model of HIV infection, HIV-infected cells had decreased ability to dethiolate PSSG (*14*). Several PSSG have been found in human lens during cataract formation, but even very young lenses contain *S*-glutathionylated α- and ß-crystallin, the reaction sites of which have been identified (*4*).

Many proteins undergo oxidant-mediated regulation by *S*-glutathionylation/deglutathionylation of functionally sensitive cysteine residues, including the 20 S proteasome, thioredoxin, glutaredoxin, 1-CYS peroxiredoxin (*7*), components of signal transduction pathways, such as protein tyrosine phosphatase-1B, H-Ras, and several protein kinase C isoenzymes (*7, 9*). The reversible *S*-glutathionylation of actin may be a physiologically-relevant regulatory mechanism of actin polymerisation (*8, 15, 16*). Several metabolic enzymes, including carbonic anhydrase III (*17, 18*), tyrosine hydroxylase, α-ketoglutarate dehydrogenase, creatine kinase (*7*), and glyceraldehyde phosphate dehydrogenase (7, *14, 19*), also appear to be regulated by *S*-glutathionylation. *S*-Glutathionylation of the cardiac heat shock protein HSP27 under oxidant stress disaggregates and inactivates it independently of phosphorylation (*20*), whereas *S*-glutathionylated HSP70 shows enhanced activity (*21*).

S-Glutathionylation is crucial in the redox regulation of HIV-1, whose protease contains conserved and critical Cys residues that, when glutathionylated, activate or deactivate the enzyme depending on the -SH group involved (*7*). *S*-Glutathionylation alters the function of proteins including ubiquitin conjugating enzymes (*22*), NF-κB/p50, c-*Jun*, protein kinase Cα, and H-ras (*7*) that are involved in cacinogenesis and other human diseases.

A mechanism by which *S*-glutathioylation regulates the kinase activity of MEKK1 [MAPK/ERK (extracellular-signal-regulated kinase) kinase kinase; MAP3K] in response to oxidative stress has been described (*23*). As MEKK1 provides a cell-survival signal, its modification may shift the balance from cell survival to cell death by apoptosis.

S-Glutathionylation may be a means of storing GSH and of cellular protection under conditions of oxidative stress, by preventing irreversible oxidation of PSH often [i.e., when the modified PSH is functionally critical (*7, 19*)] at the expense of temporary loss in activity (*2, 9, 18*). Thus, *S*-glutathionylation of: (i) γ-glutamyl transpeptidase appears to protect this membrane-bound enzyme from irreversible damage by the hydrogen peroxide produced during the enzyme involvement in metabolism of GSH, and (ii) of α-ketoglutarate dehydrogenase produces reversible inactivation in response to changes in mitochondrial GSH status (*7*).

S-Glutathionylation of Cu/Zn-superoxide dismutase, thioredoxin, and glutaredoxin (*7*) may have a direct effect on oxidant production and antioxidant defences. Furthermore, a reversible increase in production of $O_2^{\cdot -}$ by mitochondrial NADH-ubiquinone oxidoreductase results from its glutathionylation/deglutathionylation (*7*).

The key question concerning the significance of PSSG in human disease is whether they have significant consequence on protein function that affects tissue injury and/or disease progression, or are simply biomarkers for oxidative stress status. In other words, is *S*-glutationylation a cause, or a result, of a particular disease process? To answer this, it is important to show that *S*-glutathionylation actually produces altered function, and that there is a positive correlation between altered protein function and development of disease. Some studies suggest that oxidized proteins may be a link between increased oxidative stress and production of disease (*1*). Most findings have arisen from *in vitro* treatment of cells/tissues or purified proteins with oxidants, thus showing only that a protein can undergo *S*-glutathionylation, and that such modification can alter protein function(s). Direct evidence of PSSG occurrence *in vivo* has been reported in only a few papers (*1*).

Antibodies against PSSG (*8, 15, 16*) allow for redox proteomic analyses of tissue samples. This permits investigation of molecular mechanisms of pathogenesis and may help overcome the challenges of detection of oxidized proteins associated with human disease (*1*). Measurements of PSSG need to be compared with accepted markers of oxidative stress, such as protein carbonyls and nitrated and halogenated tyrosine residues. Furthermore, physiological levels of PSSG should be defined by different research groups for normal tissue, cells, or body fluids, because of the potential for artifacts in estimates of basal levels of RONS-modified proteins as a result of sample handling, processing, and analysis. This issue has plagued investigations of many different biomarkers of oxidative stress status (*1*).

Could *S*-glutathionylation of specific proteins provide a meaningful assessment of risk for development of pathology associated with increased RONS in human disease? Most RONS-induced protein modifications, e.g., protein carbonylation, are irreversible and can result in changes in protein structure and activity that may have lasting detrimental effects on cells and tissues (*1, 24*) as a result of degradation of the modified proteins or their accumulation as cytoplasmic inclusions, as occurs in neurodegenerative diseases such as AD and PD (*1, 24*). However, being reversible, *S*-glutathionylation may function as a redox switch for control of function of key proteins or to protect against the effects of oxidant stress (*2, 9*). Thus *S*-glutathionylation may function in redox signalling and in protecting proteins against irreversible oxidative modification. Possible fates of oxidized proteins are indicated in Fig. 1. Because PSSG formation is among the earliest cellular responses to an increase in RONS production, and may serve for activation/inactivation of proteins, PSSG levels may reflect the degree of oxidative stress within cells and tissues. However, the potential role of PSSG formation in normal and pathological conditions and whether this modification is protective or detrimental remain to be determined.

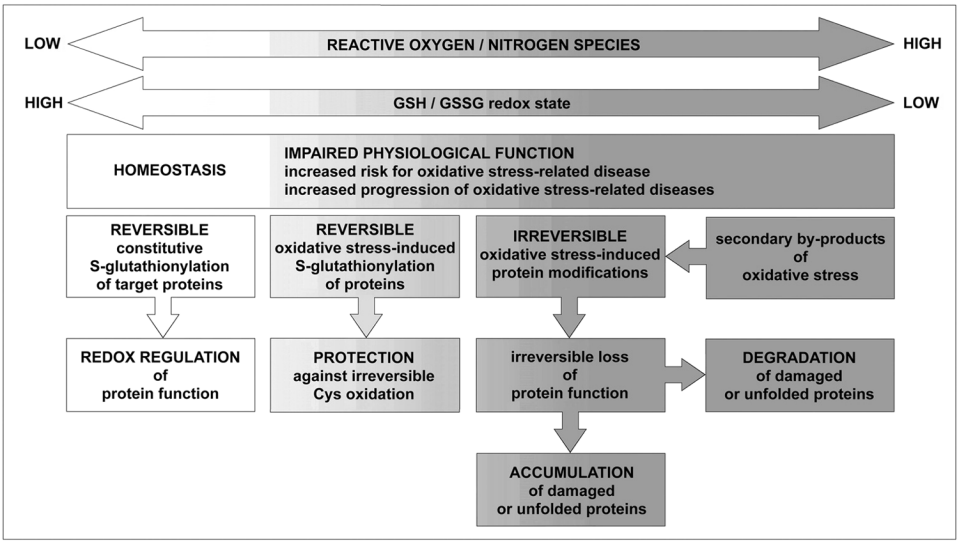

Figure 1. Possible fates of oxidised proteins. Excessive production of RONS (oxidative/nitrosative stress) may cause modifications on sensitive proteins. Reversible modifications, such as *S*-glutathionylation, may have a dual role of redox modulation of protein function and protection of PSH from irreversible oxidation. Irreversible modifications, such as protein carbonylation, are usually associated with permanent loss of function and may lead to elimination or accumulation of the damaged proteins and may contribute to several human pathologies.

ACKNOWLEDGEMENTS

Preparation of this article was supported in part by FIRST 2004 (Fondo Interno Ricerca Scientifica e Tecnologica), University of Milan, and by Fondazione Monte dei Paschi di Siena. We sincerely apologize to those authors whose work we could not cite because of space restrictions.

New Questions

(1) Is there a role for S-glutathionylation of Proteins in human disease?
(2) How does the biological function of N-myristoylation differ from that of N-palmitoylation?
(3) How did the genetic code grow, and why did it stop at about 20?
(4) What are prostasomes?

REFERENCES

1. Dalle-Donne, I., Scaloni, A., Giustarini, D., Cavarra, E., Tell, G., Lungarella, G., Colombo, R., Rossi, R., and Milzani, A. (2005) Proteins as biomarkers of oxidative/nitrosative stress in diseases: The contribution of redox proteomics. *Mass Spectrom. Rev.* **24**, 55–99.
2. Schafer, F. Q., and Buettner, G. R. (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med* **30**, 1191–1212.
3. Pastore, A., Federici, G., Bertini, E., and Piemonte, F. (2003) Analysis of glutathione: implication in redox and detoxification. *Clin. Chim. Acta* **333**, 19–39.
4. Craghill, J., Cronshaw, A. D., and Harding, J. J. (2004) The identification of a reaction site of glutathione mixed-disulphide formation on γS-crystallin in human lens. *Biochem. J* **379**, 595–600.
5. Lind, C., Gerdes, R., Hamnell, Y., Schuppe-Koistinen, I., von Lowenhielm, H. B., Holmgren, A., and Cotgreave, I. A. (2002) Identification of S-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. *Arch. Biochem. Biophys.* **406**, 229–240.
6. Pastore, A., Tozzi, G., Gaeta, L. M., Bertini, E., Serafini, V., Di Cesare, S., Bonetto, V., Casoni, F., Carrozzo, R., Federici, G., and Piemonte, F. (2003) Actin glutathionylation increases in fibroblasts of patients with Friedreich's ataxia: A potential role in the pathogenesis of the disease. *J Biol. Chem.* **43**, 42588–42595.
7. Giustarini, D., Rossi, R., Milzani, A., Colombo, R., and Dalle-Donne, I. (2004) *S*-Glutathionylation: from redox regulation of protein functions to human diseases. *J. Cell. Mol. Med.* **8**, 201–212.
8. Dalle-Donne, I., Rossi, R., Giustarini, D., Colombo, R., and Milzani, A. (2003) Actin *S*-glutathionylation: evidence against a role for glutathione disulfide. *Free Radic Biol. Med.* **35**, 1185–1193.
9. Klatt, P., and Lamas, S. (2000) Regulation of protein function by *S*-glutathiolation in response to oxidative and nitrosative stress. *Eur. J. Biochem.* **267**, 4928–4944.
10. Giustarini, D., Milzani, A., Aldini, G., Carini, M., Rossi, R., and Dalle-Donne, I. (2005) *S*-nitrosation *vs. S*-glutathionylation of protein sulfhydryl groups by *S*-nitrosoglutathione. *Antioxid. Redox Signal.* in press.
11. Eaton, P., Byers, H. L., Leeds, N., Ward, M. A., and Shattock, M. J. (2002) Detection, quantitation, purification, and identification of cardiac proteins S-thiolated during ischemia and reperfusion. *J. Biol. Chem.* **277**, 9806–9811.

12. Fratelli, M., Demol, H., Puype, M., Casagrande, S., Eberini, I., Salmona, M., Bonetto, V., Mengozzi, M., Duffieux, F., Miclet, E., Bachi, A., Vandekerckhove, J., Gianazza, E., and Ghezzi, P. (2002) Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc. Natl. Acad. Sci. USA* **99**, 3505–3510.
13. Fratelli, M., Demol, H., Puype, M., Casagrande, S., Villa, P., Eberini, I., Vandekerckhove, J., Gianazza, E., and Ghezzi, P. (2003) Identification of proteins undergoing glutathionylation in oxidatively stressed hepatocytes and hepatoma cells. *Proteomics* **3**, 1154–1161.
14. Ghezzi, P., Romines, B., Fratelli, M., Eberini, I., Gianazza, E., Casagrande, S., Laragione, T., Mengozzi, M., Herzenberg, L. A., Herzenberg, L. A. (2002) Protein glutathionylation: coupling and uncoupling of glutathione to protein thiol groups in lymphocytes under oxidative stress and HIV infection. *Mol. Immunol.* **38**, 773–780.
15. Wang, J., Boja, E. S., Tan, W., Tekle, E., Fales, H. M., English, S., Mieyal, J. J., and Chock, P. B. (2001) Reversible glutathionylation regulates actin polymerization in A431 cells. *J. Biol. Chem.* **276**, 47763–47766.
16. Dalle-Donne, I., Giustarini, D., Rossi, R., Colombo, R., and Milzani, A. (2003) Reversible S-glutathionylation of Cys(374) regulates actin filament formation by inducing structural changes in the actin molecule. *Free Radic. Biol. Med.* **34**, 23–32.
17. Cabiscol, E., and Levine, R. L. (1996) The phosphatase activity of carbonic anhydrase III is reversibly regulated by glutathiolation. *Proc. Natl. Acad. Sci. USA* **93**, 4170–4174.
18. Mallis, R. J., Hamann, M. J., Zhao, W., Zhang, T., Hendrich, S., and Thomas, J. A. (2002) Irreversible thiol oxidation in carbonic anhydrase III: protection by S-glutathiolation and detection in aging rats. *Biol. Chem.* **383**, 649–662.
19. Eaton, P., Wright, N., Hearse, D. J., and Shattock, M. J. (2002) Glyceraldehyde phosphate dehydrogenase oxidation during cardiac ischemia and reperfusion. *J. Mol. Cell. Cardiol.* **34**, 1549–1560.
20. Eaton, P., Fuller, W., and Shattock, M. J. (2002) S-thiolation of HSP27 regulates its multimeric aggregate size independently of phosphorylation. *J. Biol. Chem.* **277**, 21189–21196.
21. Hoppe, G., Chai, Y. C., Crabb, J. W., and Sears, J. (2004) Protein *S*-glutathionylation in retinal pigment epithelium converts heat shock protein 70 to an active chaperone. *Exp. Eye Res.* **78**, 1085–1092.
22. Obin, M., Shang, F., Gong, X., Handelman, G., Blumberg, J., and Taylor, A. (1998) Redox regulation of ubiquitin-conjugating enzymes: mechanistic insights using the thiol-specific oxidant diamide. *FASEB J.* **12**, 561–569.
23. Cross, J. V., and Templeton, D. J. (2004) Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP-binding domain. *Biochem J.* **381**, 675–683.
24. Dalle-Donne, I., Giustarini, D., Colombo, R., Rossi, R., and Milzani, A. (2003) Protein carbonylation in human diseases. *Trends Mol. Med.* **9**, 169–176.