ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 7 & 8, 2005

Forum Editorial

Protein *S*-Thiolation: Emphasis on Cell Signaling and Gene Expression

PHILIP EATON[1] and MICHAEL J. SHATTOCK[2]

Protein oxidation in cell biology was once almost exclusively considered in terms of injury, disease, and cellular dysfunction. The rationale was that the uncontrolled, aberrant, or over production of oxidants targeted the various cellular components, including lipids, nucleic acids, and proteins, to induce an oxidative alteration. This oxidation compromised the molecular function of these biomolecules, which could ultimately irreversibly damage the cell, tissue, or organ. Oxidative stress has been implicated in most major diseases, including those of the cardiovascular system, cancer, and neurodegeneration (3).

In recent times, however, there has been a growing recognition that oxidative stress should not only be considered in the context of damage and dysfunction. Many contemporary studies show that oxidants are produced in healthy cells and are integral to normal function and in many signaling and control pathways, as reviewed in this *Forum* (7, 9, 11, 13, 14, 17). This is perhaps best highlighted by cellular oxidant concentrations rising in response to numerous neurohormonal stimuli that impact on the activity of a variety of oxidases and mitochondrial redox centers. Thus, the term oxidative *stress* may often be inappropriate, as indeed it is questionable that stress accompanies the majority of prooxidative events.

Protein cysteines are the most reactive amino acids in tissues, with the reactivity of individual thiols increasing as their pK_a decreases. This is because, at a physiological pH, a thiol with a lower pK_a will ionize and react more readily. In some cases, these reactive proteinaceous thiols are catalytically essential, and thus a common theme developed in the scientific literature suggesting that their oxidation would likely correlate with a loss of function. However, as highlighted above, prooxidative events in cells do not necessarily equate solely with injury or damage. For example, insulin or angiotensin II-stimulated oxidant production should not be considered in terms of pathologies, but primarily in terms of an integral component of their normal signaling pathway. In this issue, protein *S*-thiolation is shown to occur during the initiation of cardiac ischemic preconditioning, implicating the reversible cysteine oxidation as an important sensor and signal transducer in this form of cardioprotection (8). Therefore, nonlethal, prooxidative events also seem to be crucial in the genesis of cellular adaptation to changes in environment. However, just as aberrant phosphoregulation occurs in many disease states, the loss of redox control may also be important in the development of dysfunction.

The regulated control of oxidant production, coupled with the fact that some oxidative modifications of proteins are reversible, has led to the recognition that the posttranslational oxidative modification of proteins can regulate protein function. In terms of cysteine oxidation, a number of oxidative derivatives can be formed, including sulfenate, sulfinite, sulfonate, nitrosated, lipid-modified, as well as an array of *S*-thiolation states involving cysteine, glutathione, homocysteine, cysteinyl-glycine (5), and other small thiols. Many of these are reversible oxidations, and perhaps the best studied in this context is *S*-thiolation, especially modification by glutathione. Although there are many molecular mechanisms and determinants of protein *S*-thiolation, these factors are not fully understood at this time, an issue that requires further study and is the subject of several original articles in this *Forum* (5, 10, 12).

The fact that redox signaling via protein *S*-thiolation is the principal focus of this *Forum* highlights to some extent the acceptance of this oxidative posttranslational modification as a *bona fide* regulator of protein function. Certainly, the current burst of research in this and related fields suggests that the case is proven and these redox modifications are established players. However, to prove conclusively that regulation by *S*-thiolation is a universal and widespread cellular event may take a few more years. Proteomic methods have identified, and continue (15) to identify, novel redox-regulated proteins. This technology is now being increasingly applied to the study of posttranslational modifications of proteins, including redox alterations at cysteine residues. Consequently, a more rapid understanding of how widespread and important these redox mechanisms are may be possible, in comparison with the relatively slow appreciation of the importance of protein phosphoregulation.

In this *Forum,* the specifics and cell biology of a number of established targets of *S*-thiolation are presented, including

[1]Department of Cardiology and [2]Cardiac Physiology, Cardiovascular Division, King's College London, London, U.K.

carbonic anhydrase (12), protein kinase C (4), tyrosine hydroxylase (16), transcription factors (2), and the ryanodine receptor (1). As more proteins are identified that may be regulated by *S*-thiolation, further studies such as these are required to test the biological relevance and assess whether there is a true correlate between redox state and functional activity. It will also be important to verify that any functional correlate with redox state really does occur in cells and tissues under physiologically or pathophysiologically relevant conditions. As loss of redox homeostasis is implicated in the pathogenesis of many diseases, a fuller understanding of how proteins may be regulated by cysteine-targeted oxidation increases the likelihood of therapeutic interventions based on rational drug design. The therapeutic potential of the pharmaceutical manipulation of the redox-dependent activity of specific proteins, including protein kinase C (4) and aldose reductase (6), is highlighted in this *Forum*.

REFERENCES

1. Aracena P, Tang W, Hamilton SL, and Hidalgo C. Effects of *S*-glutathionylation and *S*-nitrosylation on calmodulin binding to triads and FKBP12 binding to type 1 calcium release channels. *Antioxid Redox Signal* 7: 870–881, 2005.
2. Banning A and Brigelius-Flohé R. NF-κB, Nrf2, and HO-1 interplay in redox-regulated VCAM-1 expression. *Antioxid Redox Signal* 7: 889–899, 2005.
3. Bharath S and Andersen JK. Glutathione depletion in a midbrain-derived immortalized dopaminergic cell line results in limited tyrosine nitration of mitochondrial complex I subunits: implications for Parkinson's disease. *Antioxid Redox Signal* 7: 900–910, 2005.
4. Chu F and O'Brian CA. PKC sulfhydryl targeting by disulfiram produces divergent isozymic regulatory responses that accord with the cancer preventive activity of the thiuram disulfide. *Antioxid Redox Signal* 7: 855–862, 2005.
5. Corti A, Paolicchi A, Franzini M, Dominici S, Casini AF, and Pompella A. The *S*-thiolating activity of membrane γ-glutamyltransferase: formation of cysteinyl-glycine mixed disulfides with cellular proteins and in the cell microenvironment. *Antioxid Redox Signal* 7: 911–918, 2005.
6. Dal Monte M, Del Corso A, Moschini R, Cappiello M, Amodeo P, and Mura U. Zofenoprilat-glutathione mixed disulfide as a specific *S*-thiolating agent of bovine lens aldose reductase. *Antioxid Redox Signal* 7: 841–848, 2005.
7. Di Simplicio P, Frosali S, Priora R, Summa D, Cherubini Di Simplicio F, Di Giuseppe D, and Di Stefano A. Biochemical and biological aspects of protein thiolation in cells and plasma. *Antioxid Redox Signal* 7: 951–963, 2005.
8. Eaton P, Bell RM, Cave AC, and Shattock MJ. Ischemic preconditioning: a potential role for protein *S*-thiolation? *Antioxid Redox Signal* 7: 882–888, 2005.
9. Ghezzi P, Bonetto V, and Fratelli M. Thiol–disulfide balance: from the concept of oxidative stress to that of redox regulation. *Antioxid Redox Signal* 7: 964–972, 2005.
10. Giustarini D, Milzani A, Aldini G, Carini M, Rossi R, and Dalle-Donne I. *S*-Nitrosation versus *S*-glutathionylation of protein sulfhydryl groups by *S*-nitrosoglutathione. *Antioxid Redox Signal* 7: 930–939, 2005.
11. Hurd TR, Costa NJ, Dahm CC, Beer SM, Brown SE, Filipovska A, and Murphy MP. Glutathionylation of mitochondrial proteins. *Antioxid Redox Signal* 7: 999–1010, 2005.
12. Kim G and Levine RL. Molecular determinants of *S*-glutathionylation of carbonic anhydrase 3. *Antioxid Redox Signal* 7: 849–854, 2005.
13. Lockwood TD. The transfer of reductive energy and pace of proteome turnover: a theory of integrated catabolic control. *Antioxid Redox Signal* 7: 982–998, 2005.
14. Ogawa K. Glutathione-associated regulation of plant growth and stress responses. *Antioxid Redox Signal* 7: 973–981, 2005.
15. Rouhier N, Villarejo A, Srivastava M, Gelhaye E, Keech O, Droux M, Finkemeier I, Samuelsson G, Dietz KJ, Jacquot J-P, and Wingsle G. Identification of plant glutaredoxin targets. *Antioxid Redox Signal* 7: 919–929, 2005.
16. Sadidi M, Geddes TJ, and Kuhn DM. *S*-Thiolation of tyrosine hydroxylase by reactive nitrogen species in the presence of cysteine or glutathione. *Antioxid Redox Signal* 7: 863–869, 2005.
17. Shackelford RE, Heinloth AN, Heard SC, and Paules RS. Cellular and molecular targets of protein *S*-glutathiolation. *Antioxid Redox Signal* 7: 940–950, 2005.

Address reprint requests to:
Philip Eaton, Ph.D.
Department of Cardiology
Cardiovascular Division
The Rayne Institute
St. Thomas' Hospital
London, SE1 7EH, U.K.

E-mail: philip.eaton@kcl.ac.uk