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COMMENTARY

Redox Signaling and the Emerging Therapeutic Potential of Thiol Antioxidants

*Chandan K. Sen**

DEPARTMENT OF MOLECULAR AND CELL BIOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY, CA 94720-3200, U.S.A.

ABSTRACT. Oxidation-reduction (redox) based regulation of signal transduction and gene expression is emerging as a fundamental regulatory mechanism in cell biology. Electron flow through side chain functional CH_2-SH groups of conserved cysteinyl residues in proteins account for their redox-sensing properties. Because in most intracellular proteins thiol groups are strongly "buffered" against oxidation by the highly reduced environment inside the cell, only accessible protein thiol groups with high thiol-disulfide oxidation potentials are likely to be redox sensitive. The list of redox-sensitive signal transduction pathways is steadily growing, and current information suggests that manipulation of the cell redox state may prove to be an important strategy for the management of AIDS and some forms of cancer. The endogenous thioredoxin and glutathione systems are of central importance in redox signaling. Among the thiol agents tested for their efficacy to modulate cellular redox status, *N*-acetyl-L-cysteine (NAC) and α-lipoic acid hold promise for clinical use. A unique advantage of lipoate is that it is able to utilize cellular reducing equivalents, and thus it harnesses the metabolic power of the cell to continuously regenerate its reductive vicinal dithiol form. Because lipoate can be readily recycled in the cell, it has an advantage over *N*-acetyl-L-cysteine on a concentration:effect basis. Our current knowledge of redox regulated signal transduction has led to the unfolding of the remarkable therapeutic potential of cellular thiol modulating agents. BIOCHEM PHARMACOL **55**;11:1747–1758, 1998. © 1998 Elsevier Science Inc.

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Recent works from several laboratories have led to the unfolding of one of the most exciting areas in biomedical research—antioxidant and redox regulation of molecular biology. In contrast to the conventional idea that reactive oxygen is mostly a trigger for oxidative damage of biological structures, we now know that a low, physiological concentration of reactive oxygen species can regulate a variety of key molecular mechanisms that may be linked with important processes such as immune response, cell–cell adhesion, cell proliferation, inflammation, metabolism, aging, and cell death. Oxidation-reduction (redox) based regulation of gene expression appears to be a fundamental regulatory mechanism in cell biology. The primary objective of this work is to present an overview of our current understanding of redox-regulated molecular biology and to explore, in light of that knowledge, the emerging potential of thiol antioxidants.

REDOX SENSOR PROTEINS

Low levels of ROS† are generated as an integral component of normal cellular function. Under certain conditions, e.g. the presence of elevated concentrations of transition metal (Fe/Cu) ions, drug metabolism, or ischemia–reperfusion, ROS generation is exaggerated to an extent that overwhelms cellular antioxidant defenses. The result is oxidative stress. Oxidative stress has been characterized by the assessment of oxidative damage to cellular components, e.g. protein, lipid, and nucleic acid. It is now clear, however, that several biological molecules that are critically important in cell signaling and in the regulation of gene expression are sensitive to ROS at a concentration much below that required to inflict oxidative damage. Thus, much of the current focus has been directed towards the understanding of "redox sensors" in biology. A list of redox-sensitive molecular targets is presented in Table 1.

Several proteins, with apparent redox-sensing activity, have been described. Electron flow through side chain functional CH_2-SH groups of conserved cysteinyl residues in these proteins account for the redox-sensing properties.

* Correspondence: Chandan K. Sen, Ph.D., 251 Life Sciences Addition, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200. Tel. (510) 642-4445; FAX (510) 642-8313; E-mail: cksen@socrates.berkeley.edu.

† *Abbreviations:* AP-1, activator protein-1; Egr-1, early growth response-1; erTRX, exogenous recombinant human thioredoxin; GSNO, *S*-nitrosoglutathione; IL, interleukin; JNK, Jun N-terminal kinases; NAC, *N*-acetyl-L-cysteine; PDI, protein disulfide isomerase; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; TGF, transforming growth factor; TNF, tumor necrosis factor; and UVB, ultraviolet B.

TABLE 1. Redox-sensitive molecular targets

Redox-sensitive target	Characteristics/Function	Reference
Transcription factors		
NF-κB	Viral (HIV) activation, cell adhesion, NO synthesis, cytokine release, inflammation, pathogen response	[1–3]
AP-1	Cell proliferation, cell adhesion, GST regulation, multidrug resistance	[1, 3]
Elk-1	Serum response element induction, c-fos expression, cell proliferation, Ras-signaling	[4]
Egr	Osteoblast growth, herpes simplex viral activation, tumorigenesis, radiosensitivity	[5, 6]
PEBP2	Osteogenesis, muscle differentiation, T cell receptor gene arrangement, myeloperoxidase gene regulation	[7, 8]
Sp-1	HIV, herpes simplex activation, myocyte differentiation, VEGF, hsp70 and HGH gene regulation	[9]
NF-AT	IL-2 expression, IL-4 transcription, Ca^{2+} signaling, T cell activation	[10]
NF-Y	Hepatitis B viral activation, multidrug resistance, aldehyde dehydrogenase 2, and FAS gene regulation	[11]
HIF-1	Hypoxia-induced gene expression, transferrin expression, angiogenesis, tumor growth, NO synthesis	[12]
HSF	Heat-shock protein expression	[13]
Ah receptor/Arnt	Xenobiotic/pollutant response, HIF response, CYP1A1 regulation	[14]
GABP	Expression of nuclear encoded mitochondrial proteins involved in oxidative phosphorylation	[15]
TTF-1	Thyroglobulin and thyroperoxidase expression, epithelial cell gene expression in lung	[16]
PAX-8	Thyroglobulin and thyroperoxidase expression, tissue morphogenesis, neural cell adhesion, tumorigenesis	[16]
Antioxidant		
Thiol:disulfide oxidoreductases	For example, glutaredoxin, GSSG reductase, thioredoxin and thioredoxin reductase. Participate in the regulation of several redox-sensitive signaling processes, reactive oxygen scavenging, and oxidative damage repair	[17, 18]
Glutathione peroxidase	Glutathione-dependent detoxification of peroxides	[19]
Mn-SOD	Dismutation of superoxide anion radicals in the mitochondria	[20]
Calcium metabolism		
Mitochondrial permeability transition	Regulation of cytosolic [Ca^{2+}]	[21]
Adenylyl cyclase	cAMP/protein kinase A pathway, G protein effect, ion transport and hypertension, NO synthesis	[22]
Ryanodine receptor	Ryanodine binding Ca^{2+} release channels	[23]
L-Type calcium channel	Voltage dependent, form highly selective pores for Ca^{2+} in the membranes of excitable cells	[24]
Other ion transporters		
Small Cl^- channel	Gating of Ca^{2+}-dependent Cl^- channel in skeletal muscle	[25]
K^+ influx	Skeletal muscle irritability, fatigue	[26]
Cytokines		
TNF	Lymphokine with tumor necrosis activity, ROS production, programmed cell death	[27]
IL-1, -2, -6, -8	Cell proliferation, programmed cell death, T cell differentiation, inflammation	[27, 28]
TGFβ	Injury response, wound healing, cell proliferation, osteogenesis, regulation of differentiation	[29]
Cell growth-related genes		
p21	Cell cycle, terminal differentiation, programmed cell death, Ras/G protein signaling	[30, 31]
Ras-signaling	Superoxide intermediate identified to be implicated in cell proliferation	[32]
Kinase		
JNK/SAPK	MAPK-related protein kinases, cell growth and differentiation, programmed cell death, DNA repair	[33, 34]
BMK1 or ERK5	H_2O_2-sensitive MAPK, activated by MEK5	[35]

(*continued*)

TABLE 1 *(continued)*

Redox-sensitive target	Characteristics/Function	Reference
p44 MAPK/ERK1/ERK2	Aortic smooth muscle cell proliferation, CREB phosphorylation, Na^+/H^+ exchanger regulation	[36–39]
CDK	Cell cycle progression	[40]
$p56^{lck}$	Protein tyrosine phosphorylation, T cell receptor mediated signaling	[41]
Phosphatase		
Tyrosine phosphatase	Protein phosphotyrosine dephosphorylation	[42]
Other proteins		
IRP1/IRP2	Cytosolic RNA-binding proteins that bind to specific stem-loop structures termed iron-responsive elements, sensor of oxygen and iron levels	[43–45]
Glucocorticoid hormone	Endocrine function	[46]
GLUT	Glucose transport	[47]
NMDA receptor	Neuronal ion transport	[48–51]
Heme oxygenase	Catalyzes rate-limiting step of heme catabolism producing bilirubin and biliverdin, heat-shock protein	[52]
Hsp 70	Stress protein with possible antioxidant and other defense functions	[53]
Aconitase	Sensor of steady-state O_2 levels occurring in living cells and mitochondria under stress conditions	[54]

Abbreviations: Ah, aryl hydrocarbon; Arnt, Ah receptor nuclear translocator; AP, activator protein; BMK, big mitogen-activated protein kinase; CDK, cyclin-dependent kinase; CREB, cAMP regulatory element binding protein; CYP, cytochrome P450; egr, early growth response; ERK, extracellular signal-regulated kinases; FAS, fatty acid synthase; GABP, GA (purine-rich-repeat) binding protein, also known as nuclear respiratory factor 2; GLUT, glucose transporter; GSSG, glutathione disulfide; HGH, human growth hormone; HIF-1, hypoxia inducible factor 1; HSF, heat-shock factor; Hsp, heat-shock protein; IL, interleukin; IRP, iron-responsive-element-binding protein; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinases; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; NF-Y, sequence-specific DNA-binding protein (nuclear factor) that interacts with the conserved Y motif or Y box of the major histocompatibility complex class II gene, E alpha; NMDA, *N*-methyl-D-aspartate; PAX, thyroid-enriched proteins with paired-box domains for DNA interaction; PEBP2, polyomavirus enhancer binding protein 2; ROS, reactive oxygen species; SOD, superoxide dismutase; SAPK, stress-activated protein kinase; Sp-1, promoter (simian virus 40) specific factor; TGF, transforming growth factor; TNF, tumor necrosis factor, TTF, thyroid specific transcription factor; and VEGF, vascular endothelial growth factor. Selected literature demonstrating redox sensitivity is cited.

From *in vitro* information presented thus far, this mechanism appears to account for most of the major redox-driven signal transduction. It has been shown that formation of protein-disulfide bonds following oxidant challenge may lead to protein destabilization and exposure of hydrophobic domains. Such changes have been suggested to signal for oxidative stress-induced heat shock response [53]. Most intracellular protein thiol groups are strongly "buffered" against oxidation by the highly reduced environment inside the cell mediated by high amounts of glutathione, thioredoxin, and associated systems. Thus, only accessible protein thiol groups with high thiol-disulfide oxidation potentials are likely to be redox sensitive. A well-characterized redox-sensitive step in the regulation of AP-1 transcription factor is the DNA binding of Fos and Jun proteins [55]. Fos and Jun DNA binding *in vitro* is regulated by the reduction-oxidation of a single conserved cysteine residue (Lys-Cys-Arg) in the DNA-binding domains of the two proteins. The requirement of a single cysteine residue and the sensitivity of Fos and Jun proteins to the -SH alkylating agent *N*-ethylmaleimide exclude the possibility that oxidation of the cysteine residue involves intra- and intermolecular disulfide bond formation. It has been suggested that conversion of the cysteine to reversible oxidation products such as sulfenic (RSOH) or sulfinic (RSO_2H) acids could contribute to the regulation of DNA binding [55]. Replacement of the critical cysteine residue of a truncated Fos protein by serine resulted in a three-fold increase in AP-1 DNA binding activity that was no longer redox regulated. Such observations indicate that redox regulation of AP-1 DNA binding limits the total level of Fos-Jun *in vivo* and that escape from this control enhances transforming activity [56].

In NF-κB proteins, the highly conserved Rel homology domain is responsible for DNA binding. A short stretch of amino acids (the RXXRXRXX**C** motif, R = arginine, C = cysteine, X = other amino acid) at the beginning of the domain is essential to contact DNA directly [57–59]. The cysteine residue in the motif is critical and must be maintained in a reduced state to allow DNA binding because oxidation of this cysteine residue interferes with DNA binding of NF-κB [57–61]. The 128-amino acid long, evolutionarily conserved Runt domain of the alpha subunit of the transcription factor PEBP2/CBF is responsible for both DNA binding as well as heterodimerization with the regulatory subunit beta. The Runt domain contains two conserved cysteinyl residues, Cys-115 and Cys-124, that confer redox sensitivity to DNA binding of the proteins. Substitution of Cys-115 by serine partially impaired DNA binding. Substitution of Cys-124, however, increased DNA binding. Thus, it was evident that both cysteine residues were responsible for the redox regulation in their own way [8]. Recently, a molecular redox switch has been identified on p21 (ras) [31]. The Cys-118 residue containing a fragment of p21 was observed to be the critical site of redox regulation. S-Nitrosylation of this residue triggers guanine nucleotide exchange and downstream signaling [31].

Thiol:disulfide oxidoreductases, e.g. glutaredoxin, GSSG

reductase, thioredoxin, and thioredoxin reductase have active dithiol moieties and are known to play a central role in redox-sensitive signal transduction. These enzymes also provide a primary defense mechanism for protection and repair of protein sulfhydryls in an oxidative stress situation. An active-site CXXC motif of such oxidoreductases has been observed to be essential for their catalysis of redox reactions [17]. The rapid formation of native disulfide bonds in cellular proteins, necessary for the efficient use of cellular resources, is catalyzed *in vitro* by PDI. The significance of this enzyme is clearly evident in *Saccharomyces cerevisiae* in which the *PDI1* gene is essential for survival. It has been observed that the presence of the CXXC motif is essential for the formation of native disulfide bonds in the cell [62]. Amino acid oxidation-dependent redox sensitivity also has been postulated for the release and activity of TGF beta-1 (TGFβ). TGFβ is a multifunctional cytokine that orchestrates response to injury via ubiquitous cell surface receptors. The cytokine is secreted as a biologically inactive complex. Oxidation of specific amino acids in the latency-conferring peptide has been suggested to lead to a conformational change in the latent complex that allows the release and biological activity of TGFβ [29].

Both *in vitro* and *in vivo* evidence show that zinc-finger DNA-binding proteins, e.g. members of the Sp-1 family, are redox sensitive. An Sp-1 site-mediated hyperoxidative repression of transcription from promoters with essential Sp-1 binding sites, including simian virus 40 early region glycolytic enzyme, and dihydrofolate reductase genes, has been observed [9]. Binding of the transcription factor Egr-1 to its specific DNA-binding sequence GCGGGGGCG occurs through the interaction of three zinc finger motifs with demonstrated redox sensitivity [5, 6]. Proteins with iron–sulfur prosthetic groups have been identified to have remarkable redox-sensing properties. The assembly and disassembly of [4Fe-4S] clusters is the key to redox sensing in these proteins [43]. The Fe-S containing proteins acquire their clusters by post-translational assembly under the direction of L-cysteine/cystine C-S-lyase activity [63].

Oxidative stress-induced tyrosine phosphorylation has been ascribed to the activation of phosphotyrosine kinase or to inhibition of phosphotyrosine phosphatase. Reactive cysteinyl residues in the active site of protein-tyrosine phosphatases confer oxidant sensitivity to the activity of these enzymes [64]. This family of enzymes feature an essential nucleophilic thiol group that attacks the phosphorus atom in a substrate. The nucleophilic attack by Cys-12 in low molecular weight phosphotyrosine phosphatase is carried out by a thiolate anion form of this residue [65]. It has been shown that a single S to O atom substitution in the nucleophile, via Cys to Ser mutation, results in structural/conformational and functional changes that render phosphotyrosine phosphatases catalytically inactive [66]. In vitro studies [67–69] show that comparable to vanadate, hydrogen peroxide selectively inhibits phosphotyrosine phosphatase activity. Treatment of erythrocytes with the thiol-oxidizing agent diamide has been shown to lead to the formation of phosphotyrosine phosphatase disulfides [42]. Such inactivation of the enzyme inhibits dephosphorylation and results in the accumulation of phosphorylated protein tyrosine [64].

Ca^{2+}-driven protein phosphorylation and proteolytic processing of proteins are two major intracellular events that are implicated in signal transduction from the cell surface to the nucleus. Intracellular calcium homeostasis is regulated by the redox state of cellular thiols [70]. For example, the calcium release channel/ryanodine receptor complex of skeletal muscle sarcoplasmic reticulum has been shown to contain reactive thiols that are sensitive to glutathione [23]. In addition, the presence of an allosteric thiol-containing redox switch on the L-type calcium channel subunit complex has been indicated [24]. Thus, changes in the cellular thiol redox state are expected to influence calcium-sensitive signaling processes [2, 3, 24, 71].

THE KEY PLAYERS IN REDOX REGULATION

The ubiquitous endogenous thiols thioredoxin and glutathione are of central importance in redox signaling [72, 73].

The Thioredoxin System

Thioredoxin is a pleiotropic NADPH-dependent disulfide oxidoreductase that catalyzes the reduction of exposed protein S—S bridges. Because of its dithiol/disulfide exchange activity, thioredoxin determines the oxidation state of protein thiols. This small (~12 kDa) protein is evolutionarily conserved between prokaryotes and eukaryotes from yeast to animals and plants. A characteristic feature of most thioredoxins is the presence of a conserved catalytic site Trp-Cys-Gly-Pro-Cys-Lys in a protrusion of the three-dimensional structure of the protein. The two cysteine residues of the site can be reversibly oxidized to form a disulfide bridge and, thereafter, be reduced by action of the selenoenzyme thioredoxin reductase in the presence of NADPH {$NADPH + H^+ + \text{thioredoxin-}S_2 \rightarrow NADP^+ + \text{thioredoxin-}(SH)_2$}. Thioredoxin reductase activity is decreased by selenium deficiency [74]. Thioredoxin reductase from human placenta reacts with only a single molecule of NADPH, which leads to a stable intermediate similar to that observed in titrations of lipoamide dehydrogenase or glutathione reductase. Experiments related to the titration of thioredoxin reductase from human placenta with dithionite suggested that the penultimate selenocysteine of the protein is in redox communication with the active site disulfide/dithiol [75]. In addition to the two active site cysteine residues indicated above, two or three additional structural cysteine residues exist in the C-terminal half of the thioredoxin molecule. Oxidation of these residues results in a loss of the enzymatic activity of thioredoxin [76].

Thioredoxin peroxidase is a cytosolic protein that catalyzes the conversion of hydroperoxide and alkyl hydroperoxides into water and corresponding alcohols. Originally,

thioredoxin peroxidase was identified as thiol-specific antioxidant or protector protein from yeast [77]. During the course of antioxidant protection, thiols (RSH) react with free radical species ($A^{\bullet}$) to neutralize (AH) the radical. As a result of such reaction, thiyl radicals ($RS^{\bullet}$) are generated. Thiyl radicals are capable of triggering oxidative damage to biological macromolecules, e.g. lipids and DNA. It appears that thioredoxin peroxidase detoxifies thiyl radicals or oxidized thiyl radical anions [78]. Antioxidant properties of thioredoxin peroxidase also include the removal of hydrogen peroxide by the overall reaction: $2\ RSH + H_2O_2 \rightarrow RSSR + H_2O$ [79].

Mammalian thioredoxin [80] acts as a hydrogen donor for ribonucleotide reductase [81] and methionine sulfoxide reductase, facilitates refolding of disulfide containing proteins [82], activates glucocorticoid or interleukin-2 receptors [83, 84], and activates partially folded malate dehydrogenase [85]. Thioredoxin also has been shown to regulate the DNA binding activity of some transcription factors either directly, as for TFIIIC, BZLF1, and NF-κB [61, 86, 87], or indirectly as for the DNA binding of AP-1 proteins. Reduction of a single conserved cysteine residue, located in the DNA-binding domain of AP-1 proteins, by Ref-1, which in turn is reduced by thioredoxin, is required for AP-1 DNA binding activity [55]. A recent report shows that during the course of phorbol 12-myristate 13-acetate-induced activation of AP-1, thioredoxin is efficiently translocated into the HeLa cell nucleus where Ref-1 is located. This process seemed to be essential for AP-1 activation by redox modification because co-overexpression of thioredoxin and Ref-1 in COS-7 cells potentiated AP-1 activity only after thioredoxin was transported into the nucleus in response to phorbol 12-myristate 13-acetate treatment. It has been directly shown that thioredoxin can physically associate with Ref-1 in the nucleus [88]. Translocation of thioredoxin molecules from the cytoplasm to the nucleus also has been observed in response to oxidative stress conditions [89], e.g. ultraviolet irradiation [90]. Such translocation response suggests a possible role of thioredoxin in sensing and transducing oxidative stress signals [1].

Thioredoxin, secreted by cells using a leaderless pathway [91–93], stimulates the proliferation of lymphoid cells, fibroblasts, and a variety of human solid tumor cell lines [94–97]. It appears to function as an autocrine growth factor for human lymphoid cells immortalized by the human T-lymphotrophic virus type I or the Epstein-Barr virus. This proliferative effect of thioredoxin involves the activation of protein kinase C through its translocation to the membrane [98]. Active site cysteine replacement studies show that the redox active form of thioredoxin is necessary for eliciting growth stimulation [95]. Treatment of several cell types with thioredoxin strongly enhances the expression of various cytokines. Thioredoxin augments the phorbol ester-induced expression of cytokines, e.g. TNF, IL-1, IL-8, IL-2 and IL-2 specific transcripts. The synthesis of IL-6 is also increased by thioredoxin in a dose-dependent manner. Thus, cytokine synthesis appears to be tightly controlled by redox-dependent processes. As thioredoxin is readily secreted and taken up by cells, it may play an important role as a co-stimulatory molecule involved in immune processes [27].

Thioredoxin also has been identified as an essential component of the early pregnancy factor [99], and it is known to inhibit human immunodeficiency virus expression in macrophages [100]. Antioxidant properties of thioredoxin include removal of hydrogen peroxide [101], free radical scavenging [102], and protection of cells against oxidative stress [89, 103]. Recycling of ascorbate from its oxidized forms is essential to maintain stores of the vitamin in human cells. Previous works have shown that reduction of dehydroascorbate to ascorbate is largely GSH dependent. Recently, it has been demonstrated that the selenium-dependent thioredoxin reductase system might contribute to ascorbate regeneration. It has been observed that purified rat liver thioredoxin reductase functions as an NADPH-dependent dehydroascorbate reductase. GSH-dependent dehydroascorbate reductase activity in liver cytosol was variable, but typically 2- to 3-fold that of NADPH-dependent activity [104]. The thioredoxin system can reduce dehydroascorbate and thus may be counted in as a significant component of the antioxidant defense network [105]. Under conditions of L-cystine and glutathione depletion, the antioxidant defenses of lymphoid cells are impaired. This results in apoptosis, most likely via an oxidant-dependent mechanism. Thioredoxin has been observed to be protective under such conditions, perhaps by virtue of its antioxidant properties [106]. UVB radiation is known to induce the generation of reactive oxygen species in the skin. Thioredoxin has been shown to be efficiently produced in, and released from, cultured normal human keratinocytes after UVB irradiation. Thioredoxin released from UVB-irradiated keratinocytes acts as a survival factor for both keratinocytes and melanocytes but does not prevent UV-induced melanocyte death. Furthermore, it has been suggested that thioredoxin may work as one of the stimulatory factors for UVB-induced melanogenesis [107]. When stored in the absence of reducing agents, human recombinant thioredoxin undergoes spontaneous oxidation, losing its ability to stimulate cell growth, but is still a substrate for NADPH-dependent reduction by human thioredoxin reductase. There is a slower spontaneous conversion of thioredoxin to a homodimer that is not a substrate for reduction by thioredoxin reductase and that does not stimulate cell proliferation. Both conversions can be induced by chemical oxidants and are reversible by treatment with the thiol reducing agent dithiothreitol [108].

Interaction of NO generated in cells with thiols results in the formation of nitrosothiols. The NO-generating enzyme NO synthase itself is a target of such NO-dependent modification. Interaction of NO with vicinal dithiols in the regulatory domain of NO synthase protein is responsible for post-translational reduction of its catalytic activity. Thioredoxin has been observed to be able to reverse such NO-dependent functional inactivation of NO synthase

[109]. In activated human neutrophils, a burst of NO converts intracellular GSH to GSNO, which is subsequently cleaved to restore GSH by an unknown mechanism. It has been observed recently that GSNO is an NADPH-oxidizing substrate for human or calf thymus thioredoxin reductase. Addition of human thioredoxin stimulated the initial NADPH oxidation rate several-fold but was accompanied by progressive inactivation of thioredoxin reductase. It has been evident that thioredoxin facilitates a homolytic cleavage mechanism of GSNO, giving rise to GSH and NO [110]. This ability of the thioredoxin system to process nitrosothiols suggests novel mechanisms for redox signaling.

The Glutathione System

Glutathione has emerged to be one of the most fascinating endogenous molecules present in all animal cells, often in quite high (millimolar) concentrations. It is known to have multifaceted physiological functions including antioxidant defense, detoxification of electrophilic xenobiotics, modulation of redox-regulated signal transduction, storage and transport of cysteine, regulation of cell proliferation, synthesis of deoxyribonucleotides, regulation of immune response, and regulation of leukotriene and prostaglandin metabolism. A key mechanism that accounts for much of the metabolic and cell regulatory properties of glutathione is thiol-disulfide exchange equilibria. The function of several physiological proteins, including enzymes and signaling molecules, is regulated by thiol-disulfide exchange between protein thiols and low molecular weight disulfides. Thus, the side chain sulfhydryl (—SH) residue in cysteine of glutathione accounts for most of its physiological properties. It has been suggested that the secretion of low molecular weight thiols, e.g. cysteine and glutathione, from the endoplasmic reticulum might link disulfide bond formation in the organelle to intra- and intercellular redox signaling [111]. Protein folding in the endoplasmic reticulum often involves the formation of disulfide bonds. The oxidizing conditions required within the endoplasmic reticulum is maintained through the release of small thiols, mainly cysteine and glutathione [111].

The antioxidant function of GSH is implicated through two general mechanisms of reaction with reactive oxygen species: direct or spontaneous, and glutathione peroxidase catalyzed. As a major by-product of such reactions, GSSG is produced. Intracellular GSSG thus formed may be reduced back to GSH by glutathione reductase activity or released to the extracellular compartment. At low levels of cytosolic GSSG, T-cells fail to activate NF-κB in response to certain stimuli, whereas a high GSSG concentration inhibits the binding of activated NF-κB to its cognate DNA site. Thus, it appears that an intermediate optimal level of intracellular GSSG is required for effective NF-κB activation [72]. Droge *et al.* [72] have found that GSH deficiency of T-cells is associated with a suppression of NF-κB function. Such GSH deficiency-dependent NF-κB response is observed in certain NF-κB activation systems. For example, suppression of hydrogen peroxide-dependent NF-κB activation has been observed consistently in GSH-deficient cells [3, 112]. This effect is perhaps related to very low levels of GSSG in GSH-deficient cells. Studies with myoblasts show, however, that GSSG may participate in, but is not required for, TNFα-induced NF-κB activation. In contrast to the response of NF-κB to hydrogen peroxide, TNFα-induced NF-κB activation is potentiated in GSH-deficient cells [113]. Intracellular GSH also has been suggested to be of importance in the transcriptional activation of AP-1 and Egr-1 by a redox-dependent mechanism [114]. A role of intracellular GSH in the expression of the oncoprotein c-jun, an AP-1 family member, has been demonstrated recently [115]. Cellular GSH depletion is accompanied by decreased cell proliferation. One critical intermediate of the mitogenic cascade that appears to be sensitive to cell GSH is the function of platelet-derived growth-factor-receptor. Autophosphorylation of this receptor has been shown to be severely impaired at low glutathione levels in the cell [116]. Thiol-groups confer redox-susceptibility to the zinc-finger transcription factor Sp1, and this redox-susceptibility is prevented by DNA binding and depends on zinc coordination of the protein. It has been shown that DNA binding of apo-Sp1, but not of the holo-protein, is decreased markedly in the presence of GSH/GSSG ratios within the physiological range [117].

The involvement of intracellular Ca^{2+} in oxidant-induced NF-κB activation in T cells has been reported [2]. Because cell calcium response is known to be sensitive to thiol agents, this could be one mechanism by which thiols may modulate NF-κB activation [2, 3] or adenylyl cyclase activation [22]. In other experimental systems, the activity of the capacitative Ca^{2+} influx channel has been found to be sensitive to thiol reagents formed endogenously within the cell. Cytosolic GSSG, produced within the endothelial cell, has been shown to decrease luminal Ca^{2+} content of $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores. Depletion of internal Ca^{2+} stores by GSSG may represent a mechanism by which some forms of oxidant stress inhibit signal transduction in the vascular tissue [118].

The activation of JNK/SAPKs is a characteristic feature of stress response in several experimental systems. It has been identified recently that the intracellular GSH level plays a central role in the JNK/SAPK activation cascade. Elevation of the cellular GSH level inhibited, whereas depletion of the cellular GSH pool potentiated, induction of JNK/SAPK activity in response to an appropriate stimulus [119]. The synthesis of a number of heat-shock proteins is induced in response to various forms of environmental stress. The resultant induction of heat-shock protein gene transcription is brought about by the activation of specific transcription factors termed heat-shock factors that exist in a latent form in nonstressed cells. Depletion of cellular GSH or protein thiol oxidation triggers the activation of heat-shock factor [13]. Heme catabolic processes produce the antioxidants biliverdin and bilirubin, as well as the

potent prooxidant free iron. Since these products have opposing effects on oxidative stress, it is not clear whether heme catabolism promotes or inhibits inflammatory processes, including atherosclerotic lesion formation. Heme oxygenase, a member of the heat-shock protein family, catalyzes the rate-limiting step of heme catabolism. Endogenous glutathione levels in fibroblasts modulate both constitutive and UVA radiation/hydrogen peroxide-inducible expression of the human heme oxygenase gene [52]. Expression of inducible heme oxygenase under GSH-deficient conditions has been proposed to involve the activation of AP-1 (Jun/Jun) binding [120].

MANIPULATION OF THE CELL REDOX STATUS

Among the several thiol agents tested for their efficacy to modulate cellular redox status, NAC and α-lipoic acid hold the most promise for clinical use [121, 122]. Some fundamental criteria that such drugs should satisfy for clinical use are: a) safety, i.e. nontoxic in humans; b) elevate cell GSH; and c) favorably modulate molecular responses that are implicated in disease pathogenesis, e.g. inhibition of NF-κB in HIV infection. Both NAC and lipoate meet the above-mentioned criteria.

A common limiting factor in GSH synthesis is the bio-availability of cysteine inside the cell. In the extracellular compartment, 90% of cysteine is estimated to be present as oxidized cystine [72]. In tissue culture media, all of cysteine is present as cystine. Cells such as T lymphocytes have a weak membrane x_c^- transport system for cystine. However, the cysteine transporting ASC system is estimated to be ten times more efficient than x_c^-. Thus, delivery of the amino acid in its reduced form outside the cell should facilitate the availability of this GSH precursor inside the cell. Both NAC and lipoate facilitate cysteine delivery to the cell in their own unique ways [121].

Cysteine *per se* is highly unstable in its reduced form. As a result, considerable research has been focused on alternative strategies for cysteine delivery. In the N-acetylated form, i.e. NAC, the redox state of cysteine is markedly stabilized. After free NAC enters a cell, it is rapidly hydrolyzed to release cysteine. NAC, but not *N*-acetyl-D-cysteine or the oxidized disulfide form of NAC, is deacetylated in several tissues to release cysteine [121]. Lipoate functions as the prosthetic group for several redox reactions catalyzed by cellular α-keto-acid-dehydrogenases such as the pyruvate dehydrogenase complex. When administered to cells, lipoate is reduced rapidly to dihydrolipoate and released outside the cell. Members of the pyridine nucleotide-disulfide oxidoreductase family of dimeric flavoenzymes, e.g. lipoamide dehydrogenase, thioredoxin reductase, and glutathione reductase, reduce intracellular lipoate to dihydrolipoate in the presence of the cellular reducing equivalents NADH or NADPH. Thus, a unique advantage of lipoate is that it is able to utilize cellular reducing equivalents, and thus harnesses the metabolic power of the cell to continuously regenerate its reductive vicinal dithiol form. Because of such a recycling mechanism, the lipoate-dihydrolipoate couple can be maintained continuously in a favorable redox state at the expense of the cell's metabolic power. Dihydrolipoate released from cells reduces extracellular cystine to cysteine, and thus promotes cellular cysteine uptake via the ASC system. The dihydrolipoate/lipoate redox couple has a strong reducing power, with the standard reduction potential estimated to be −0.32 V. The ability of this couple to reduce protein thiols, e.g. thioredoxin [123, 124], has been evident, suggesting that lipoate may be effective in modulating redox-sensitive signal transduction. Redox modulatory properties and implications of both lipoate and NAC have been reviewed recently [1, 125]. The observed favorable effects of both lipoate and NAC on the molecular biology of HIV infection suggest a strong potential of these drugs for AIDS treatment [121, 122].

The therapeutic potential of erTRX also has been investigated in a few studies. erTRX inhibited the expression of human immunodeficiency virus in human macrophages (Mϕ) by 71%, as evaluated by p24 antigen production and the integration of provirus at 14 days after infection. On a concentration basis, thioredoxin was 30,000-fold more effective in inhibiting HIV production compared with the reducing agent *N*-acetylcysteine. erTRX is cleaved by Mϕ to generate the inflammatory cytokine, eosinophil cytotoxicity-enhancing factor. In contrast to the effect of thioredoxin, eosinophil cytotoxicity-enhancing factor enhances the production of HIV by 67%. Thus, whereas thioredoxin is a potent inhibitor of the expression of HIV in human Mϕ, cleavage of thioredoxin to eosinophil cytotoxicity-enhancing factor creates a mediator with the opposite effect. Thioredoxin also inhibited the expression of integrated provirus in chronically infected cells, indicating that it can act at a step subsequent to viral infection and integration [100]. Thioredoxin has been shown to be deficient in tissues but high in the plasma of AIDS patients. Approximately 25% of the HIV-infected individuals studied had plasma thioredoxin levels greater than the highest level found in controls (37 ng/mL). Interestingly, AIDS patients with higher plasma thioredoxin levels (37 ng/mL or greater) tended to have lower overall CD4 counts. In addition, an increase in plasma thioredoxin levels correlated with decreased cellular thiols and with changes in surface antigen expression (CD62L, CD38, and CD20) that occur in the later stages of HIV infection. Thus, it is apparent that elevation of plasma thioredoxin levels may be an important component of advanced HIV disease, perhaps related to the oxidative stress that is suspected to occur at this stage [126]. Thus, strategies involving modulation of the cell redox state appear to have a strong potential in the management of the HIV disease [72, 127, 128].

Human thioredoxin also contributes to cellular drug resistance. Thus, an effective strategy to sensitize cancer cells to anti-cancer drugs is to down-regulate cellular thioredoxin activity pharmacologically or by using molecular biology tools such as thioredoxin antisense constructs.

The expression and activity of thioredoxin in Jurkat cells were dose-dependently enhanced by exposure to cisplatin. Treatment of Jurkat cells with cisplatin caused transcriptional activation of the human thioredoxin gene through increased generation of intracellular reactive oxygen intermediates. Cells overexpressing exogenous human thioredoxin displayed increased resistance to cisplatin-induced cytotoxicity, compared with the control clones. After exposure to cisplatin, the control cells showed a significant increase in the intracellular accumulation of peroxides, whereas the thioredoxin-transfected cells did not. Thus, overexpressed human thioredoxin was observed to be responsible for the development of cellular resistance to cisplatin, possibly by scavenging intracellular toxic oxidants generated by this anticancer agent [129]. Thioredoxin-dependent increased resistance to Adriamycin® also has been reported. Adult T-cell leukemia cell lines expressing thioredoxin at levels 2.8 to 12 times those of other T-cell acute lymphocytic leukemia cell lines were 2–15 times more resistant to Adriamycin® than other T-cell acute lymphocytic leukemia cell lines. Diamide and sodium selenite, which have been reported to inhibit thioredoxin, restored the sensitivity to Adriamycin® in adult T-cell leukemia cell lines [130]. Nitrosoureas of the carmustine type inhibit only the NADPH reduced form of human thioredoxin reductase and thereby impair thioredoxin activity. Because these compounds are widely used as cytostatic agents, it has been suggested that thioredoxin reductase should be studied as a target in cancer chemotherapy [75]. In thioredoxin antisense transfectants, enhanced sensitivity of cancer cells to drugs such as cisplatin and also other superoxide-generating agents, e.g. doxorubicin, mitomycin C, etoposide, and hydrogen peroxide, as well as to UV irradiation, has been observed [131]. Thioredoxin also plays an important role in the growth and transformed phenotype of some human cancers. The inhibition of tumor cell growth by a dominant-negative redox-inactive mutant thioredoxin suggests that thioredoxin could be a novel target for the development of drugs to treat human cancer [132].

The distribution of thioredoxin in the brain implicates an important function in nerve cell metabolism, especially in regions with high energy demands, and indicates a role of the choroid plexus in nerve cell protection from environmental influences. After mechanical injury induced by partial unilateral hemitransection, thioredoxin mRNA expression is up-regulated in the lesioned area and spreads to the cortical hemispheres at the lesioned level. Such a response suggests a function of thioredoxin in the regeneration machinery of the brain following mechanical injury and oxidative stress [133]. Mouse thioredoxin peroxidase has a broad tissue distribution, but its expression is especially marked in cells that metabolize oxygen molecules at high levels such as erythroid cells, renal tubular cells, cardiac and skeletal muscle cells, and certain type of neurons. Levels of increased expression of thioredoxin peroxidase in the brain have been observed to be coincident with regions known to be especially sensitive to hypoxic and ischemic injury in humans. Expression of mouse thioredoxin peroxidase in PC12 pheochromocytoma cells prolonged survival of the cells in the absence of nerve growth factor and serum, indicating that thioredoxin peroxidase is able to promote neuronal cell survival. Thus, it has been proposed that thioredoxin peroxidase contributes to antioxidant defense in erythrocytes and neuronal cells by limiting the destructive capacity of oxygen radicals [134]. These findings have identified a novel gene that appears to be relevant to hypoxic brain injury and may be of importance in the development of new approaches to abrogate the effects of ischemic- and hypoxic-related injury in the central nervous system.

This work is dedicated to the memory of my loving father, Dulal C. Sen, who passed away at the age of 58 in August 1997.

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