Oxidant Sensing by Reversible Disulfide Bond Formation

Claudia M. Cremers¹ and Ursula Jakob¹,²

¹From the Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109
²Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

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To whom correspondence should be addressed: Ursula Jakob, Department of Molecular, Cellular and Developmental Biology, 830 N. University Ave., Ann Arbor, MI 48109, USA, Tel.: (734) 615-1286; Fax: (734 647 0884); E-mail: ujakob@umich.edu

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Maintenance of the cellular redox balance is crucial for cell survival. An increase of reactive oxygen, nitrogen, or chlorine species can lead to oxidative stress conditions, potentially damaging DNA, lipids, and proteins. Proteins are very sensitive to oxidative modifications, particularly methionine and cysteine residues. The reversibility of some of these oxidative protein modifications makes them ideally suited to take on regulatory roles in protein function. This is especially true for disulfide bond formation, which has the potential to mediate extensive, yet fully reversible structural and functional changes, rapidly adjusting the protein’s activity to the prevailing oxidant levels.

Cysteine: A Highly Versatile Amino Acid

Cysteine residues can take on a diverse set of roles that affect the structure, function, and regulation of proteins. They serve as metal-coordinating residues in countless Zn- or Fe-S cluster proteins, work as nucleophilic centers in the active site of metabolic enzymes and oxidoreductases, and can form covalent bonds with other cysteines (1-6). This high versatility is in large part due to the reactivity of the cysteine’s thiol group, which is affected and can be manipulated by the local protein environment. The high reactivity of cysteine thiols also contributes to the fact that cysteines are one of the least abundant amino acids in proteins and, when present, are often very highly conserved and functionally and/or structurally important (7). Although the percentage of cysteines in proteomes increases with the complexity of the organism (from ~0.4% in certain archea to ~2.25% in mammals), it is still significantly lower than the expected 3.28%, which is the probability of cysteine occurrence simply based on its codon usage (8, 9). In addition, bioinformatic analysis reveal that cysteine residues follow a distinct pattern of conservation (10). Whereas surface-exposed cysteines typically show very poor conservation scores, cysteines that occur in clusters have very high conservation scores. This result agrees with experimental data showing that cysteines that confer redox activity or coordinate metals are often found in very close proximity (10, 11), a feature that has been widely used in prediction programs seeking to identify redox-sensitive proteins in silico (12, 13).

What makes cysteine residues so special? Cysteines contain a polarizable sulfur atom, and their oxidation state can range from the fully reduced thiol/thiolate anion to the fully oxidized sulfonic acid (Fig. 1). Most reactions of thiols in biochemical systems involve the nucleophilic attack of the deprotonated thiolate anion (RS⁻) on an electrophilic center, hence making the overall reactivity of a thiol group strongly dependent on the pKₐ value of the cysteine side chain. In free cysteine, the pKₐ of the thiol is 8.3–8.5, indicating that it is almost fully protonated at physiological pH (14). In proteins, pKₐ values of cysteine thiols are dramatically influenced by their local environment. While the pKₐ values of cysteines buried in the core are on average ~9.5 (10), values...
as low as 3.4 have been reported for some active site cysteines (15). Low pK\textsubscript{a} values appear to be primarily due to stabilizing hydrogen bonds between the cysteine sulfur and the polypeptide backbone or nearby side chains — the higher the number of stabilizing hydrogen bonds, the lower the pK\textsubscript{a} of the cysteine (16-18). Positively charged amino acids in close vicinity to the cysteine, which have long been thought to play a major role in stabilizing the deprotonated thiolate anion (15, 19), seem to take on a smaller role in reducing the cysteine pK\textsubscript{a}, unless hydrogen bonds are involved as well (20). Helix dipoles also appear to contribute to a decrease in the pK\textsubscript{a} of cysteine residues, making cysteines near the N-terminal end of helices more likely to have lower pK\textsubscript{a} values than those in other parts of the protein (21).

A third parameter that substantially decreases the pK\textsubscript{a} of cysteines is the coordination of metal ions such as Zn\textsuperscript{2+} and Fe\textsuperscript{2+/3+}, which stabilize the negatively charged thiolate anions (22, 23). pK\textsubscript{a} prediction programs such as PROPKA (24) are helpful in identifying proteins with cysteine thiols of particularly low pK\textsubscript{a}. These proteins are potentially crucial players in redox-regulated processes, since the thiol reactivity might enable them to sense small alterations in the levels of endogenous reactive oxygen species (ROS) and respond with changes in gene expression, metabolism, or signaling (17, 25). It should be noted however that while the fraction of reactive thiocysteines increases with decreasing pK\textsubscript{a}-values, the reactivity of thiocysteines appears to either decrease with decreasing pK\textsubscript{a} or is unaffected by pK\textsubscript{a}, depending on the substrate tested (18).

Selenocysteine, the 21\textsuperscript{st} amino acid, is structurally very similar to cysteine apart from the selenium atom, which is in place of sulfur. This single atom change increases the reactivity of selenocysteine as compared to cysteines (26). Selenocysteines are almost exclusively found in the catalytic sites of enzymes, either performing thiol-seleno-disulfide exchange reactions or scavenging oxidants (27).

**Cysteine Thiol: A Popular Player in Redox Biology**

ROS such as superoxide (O\textsubscript{2}\textsuperscript{-}) and peroxide (H\textsubscript{2}O\textsubscript{2}) are constantly produced by members of the electron transport chain (28), NADPH oxidases (29), and in peroxisomes (30). Moreover, UVA radiation and certain xenobiotics induce endogenous ROS generation via photochemical reactions with chromophores or cytochrome P450, respectively (31, 32). In addition, organisms are exposed to high levels of exogenously produced ROS. For instance, bacteria encounter bactericidal concentrations of hypochlorous acid (HOCl) once engulfed by neutrophils (33, 34).

Whether endogenously produced or taken up by diffusion, ROS have the potential to oxidize and unfold proteins, contributing to the damaging effects of oxidative stress. In some proteins, ROS transiently modulate protein activity, most commonly via the formation of sulfenic acids that lead to disulfide bonds (see below). Reversible ROS-induced thiol modifications were originally identified as sensing mechanisms in proteins involved in oxidative stress defense. It is now becoming clear however that ROS are not simply toxic species that inevitably cause oxidative stress. Instead, ROS such as H\textsubscript{2}O\textsubscript{2} are often transiently and locally produced as part of signaling processes, reversibly modulating redox-sensitive proteins in signaling pathways. ROS-mediated signaling has recently been shown to be involved in growth, development, and differentiation processes (35), making redox-regulated disulfide bond formation an important alternative post-translational control mechanism on par with phosphorylation/dephosphorylation reactions.

**Mechanism of Disulfide Bond Formation**

When reactive cysteine thiols meet reactive oxygen, nitrogen, or chlorine species, sulfenic acid (R-SOH) formation occurs (Fig. 1). Although stabilized in protein environments that lack nearby nucleophiles, such as in NADPH peroxidases and human serum albumin (36, 37), sulfenic acids are generally considered to be highly unstable oxidation intermediates. They rapidly interact with nearby cysteines to form inter- or intramolecular disulfide bonds (R-S-S-R), making this the primary route for oxidant-mediated disulfide bond formation (38). Alternatively, sulfenic acids react with the small tripeptide glutathione (GSH), leading to S-glutathionylation. The rate with which protein thiols react with oxidants depends on the oxidant as well as on the reactivity of the respective cysteine (18, 36). While the pK\textsubscript{a} values and accessibility of the cysteines are clearly contributing factors in thiol reactivity, additional
factors, such as desolvation of reactive cysteines and steric hindrance seem to be involved as well. Moreover, substrate binding might affect a protein’s reactivity by influencing the environment of the nucleophile. For instance, caspase-3’s active site cysteine shows a significantly higher reactivity towards H$_2$O$_2$ in the presence of substrates than in the absence of client proteins (39). That other factors apart from pK$_a$ affect thiol reactivity is also illustrated by the fact that the reaction rate of protein thiols with oxidants such as H$_2$O$_2$ spans over seven orders of magnitude without any detectable correlation to the acidity of the respective active site thiol (18).

One class of enzymes that undergo oxidant-mediated disulfide bond formation within the reducing environment of the cytosol are oxidant-detoxifying enzymes, such as 2-Cys peroxiredoxins, which use reversible disulfide bond formation as a mechanism to decompose H$_2$O$_2$ and organic peroxides (40). Most other proteins that undergo reversible oxidant-mediated disulfide bond formation use this mechanism to regulate their activity in response to oxidants. These proteins are commonly referred to as redox-regulated proteins. Like peroxiredoxins, many of these proteins function in the first line of oxidative stress defense, acting as: transcriptional regulators (e.g., OxyR, Yap1p), which rapidly induce the expression of antioxidant genes to detoxify ROS and repair the damage (41, 42); chaperones (e.g., Hsp33), which prevent oxidative stress-induced protein aggregation (3, 43); or metabolic enzymes (e.g., GapDH), which use reversible oxidation to re-route metabolites, thereby increasing the cell’s capacity to defend against oxidative stress (44). Other redox-regulated proteins, such as Keap1 and PKC, are involved in signal transduction cascades (45, 46). These proteins use reversible disulfide bond formation to rapidly adapt cellular signaling processes to the oxidation status of the cell.

Oxidant-mediated disulfide bond formation, which typically occurs within the reducing environment of the cytosol, is distinct from the formation of structural disulfide bonds, which take place within the oxidizing milieu of the eukaryotic endoplasmic reticulum (ER) or the bacterial periplasm. Structural disulfide bonds are typically introduced through direct thiol-disulfide exchange reactions, catalyzed by enzymes with redox-catalytic cysteines (47-49). Notable exceptions are the Erv1/Erv2 proteins, which belong to the family of multi-domain sulfhydryl oxidases (50). These oxidases introduce disulfide bonds into client proteins in the ER and mitochondrial intermembrane space, transferring the electrons directly onto molecular oxygen, generating considerable amounts of H$_2$O$_2$ in this process (51, 52).

**Reversibility: An Important Aspect of Redox Regulation**

Specialized reductases are responsible for restoring the original redox state of cysteines (2). Members of the highly conserved Trx- and Grx-family fulfill this role in nearly all organisms studied. Like other oxidoreductases, Trx and Grx contain two redox-active cysteines arranged in a Cys-X$_2$-Cys motif, which is embedded within a characteristic thioredoxin fold (53). Thiol-disulfide exchange reactions are initiated by the more N-terminally located cysteine, which has a low pK$_a$ and attacks the disulfide bond in client proteins, forming a transient mixed disulfide intermediate in this process. This mixed disulfide bond is subsequently cleaved by a second nucleophilic attack of the more C-terminally located cysteine, resulting in the reduction of the client protein and the oxidation of the oxidoreductase (54). Oxidized Trx is then directly reduced by thioredoxin reductase, which uses NADPH for its own reduction (55). Oxidized Grx initially forms a mixed-disulfide with GSH. This mixed disulfide bond is subsequently attacked by a second nucleophilic attack of the more C-terminally located cysteine, resulting in the reduction of the client protein and the oxidation of the oxidoreductase (54). Oxidized Trx is then directly reduced by thioredoxin reductase, which uses NADPH for its own reduction (55). Oxidized Grx initially forms a mixed-disulfide with GSH. This mixed disulfide bond is subsequently attacked by a second GSH molecule, forming oxidized glutathione (GSSG) and reduced Grx (56). Glutathione reductase, in turn, reduces oxidized GSSG, also utilizing cellular NADPH as the electron donor. The major functions of Trx and Grx include the reduction of protein sulfenic acids, disulfide bonds, S-nitrosylations, and S-glutathionylations (57, 58). While the two systems are able to functionally complement each other in vivo (59), some degree of specificity has been observed for the two protein families. Thioredoxin appears to be the main system for reducing sulfenic acids. In contrast, glutaredoxin seems to be primarily responsible for the reduction of S-glutathionylated proteins (60). In this reaction, the N-terminal active site cysteine of Grx attacks the glutathionylated cysteine in client proteins, leading to the formation of glutathionylated glutaredoxin...
and the release of the reduced substrate (61). Grx is subsequently reduced by a second molecule of glutathione.

**Cysteine-coordinating Metal Centers as Redox Switches in Oxidative Stress Defense**

The ability of proteins to use reversible disulfide bond formation as a functional switch requires high reactivity of the participating cysteine thiols (*i.e.*, low pKₐ), close proximity to other cysteines, and sufficient disulfide-mediated conformational rearrangements to affect the function of the protein. An almost ideal combination of these features is found in cysteine-coordinating zinc (Zn²⁺) centers, where the positive charges of Zn²⁺ serve to stabilize the reactive thiolates and Zn²⁺ coordination enables often distant cysteines to come into very close spatial proximity. Moreover, high affinity zinc binding confers considerable stability to protein domains that would otherwise be too unstable to fold. Hence, major structural rearrangements can result from the formation of disulfide bonds and zinc release. It is therefore not surprising that zinc centers, originally thought of as redox inert and purely structural features in proteins, are now increasingly recognized as common redox units in redox-regulated proteins (3, 45) (see examples in Table S1).

One well-characterized redox-regulated protein that uses a zinc-binding motif as a redox switch is the molecular chaperone Hsp33 (Fig. 2A). Specifically activated by oxidative disulfide bond formation, Hsp33 protects organisms against the lethal consequences of oxidative stress. In the reducing, non-stress environment of the bacterial cytosol, Hsp33 is reduced, zinc-coordinated, and chaperone-inactive. Zinc binding is mediated by four tetrahedrally arranged thiolate anions, which coordinate one Zn²⁺ ion with attomolar affinity (3). Exposure of Hsp33 to fast acting oxidants, such as HOCl, leads to the rapid formation of two disulfide bonds, zinc release, and the unfolding of the C-terminal redox switch domain. Unfolding of this domain leads to the activation of Hsp33 as a chaperone (Fig. 2A) (43, 62), making it capable of binding oxidatively unfolding proteins and preventing their irreversible aggregation. Once oxidative stress conditions subside, Hsp33’s cysteines are re-reduced and zinc is re-coordinated. Refolding of Hsp33 appears to trigger the release of client proteins for refolding by the ATP-dependent chaperone system DnaK/J/GrpE, closing the activation cycle of Hsp33 (63).

It is noteworthy that incubation of Hsp33 with slow acting oxidants, such as H₂O₂, is not sufficient to activate the chaperone. Although H₂O₂ appears to be sufficient to induce formation of one disulfide bond in the distal cysteine pair, the second disulfide does not form unless unfolding conditions, such as heat shock, are present as well (5, 62). Structural studies suggest that limited accessibility of the remaining cysteines prevents H₂O₂ from forming the second disulfide bond. These results serve to illustrate that the reactivity and accessibility of cysteines can differ even within the same Zn²⁺ coordination site, conferring additional layers of regulation and oxidant specificity to zinc-mediated redox switches. The very same features, however, make predictions about the potential redox sensitivity of cysteines in zinc centers challenging and experimental evaluation on a case-by-case basis necessary.

Many other zinc-binding proteins have been shown to be sensitive to oxidation. Examples of redox-regulated eukaryotic zinc finger proteins include transcriptional regulators, such as metal regulatory transcription factor 1 (MTF-1), whose oxidative stress-mediated disulfide bond formation and homo-dimerization increases DNA binding affinity and thus transcription of target genes (64) (Table S1). Most other regulators, including specificity protein 1 (SP-1) or the tumor suppressor protein 53 (p53), use their redox responsive zinc centers directly for DNA binding. Therefore, these proteins lose transcriptional activity upon oxidative disulfide bond formation and zinc release (65-67).

Protein kinase C (PKC), a eukaryotic kinase with two zinc-binding domains, has also been shown to be redox-regulated (6). Exposure of PKC to oxidants, like H₂O₂, causes oxidation of the two regulatory zinc finger motifs, thereby relieving PKC’s auto-inhibition. This mechanism promotes activation of PKC in the absence of Ca²⁺ or phospholipids, second messengers that are otherwise necessary for its activation (6, 45, 68). Oxidative activation of PKC appears to either lead to pro-survival signaling (NFκB, Akt/ASK1) or cell death (SAPK/JNK, ERK1/2, p38), depending
on the isoform of PKC that is activated, its cellular localization, and the cell type affected (6).

**Thiol-disulfide Switches in Redox-regulated Transcription Factors**

One very well characterized member of the group of non-metal-binding redox-regulated proteins is OxyR, a prokaryotic transcription factor, whose reactive cysteine shows exquisite sensitivity to H$_2$O$_2$ (Fig. 2B). OxyR, which forms a homo-tetrameric DNA-binding structure (69), negatively regulates its own transcription and positively regulates the expression of OxyS, a small non-translated RNA, as well as approximately 30 other genes (including ahpC/F, katG) involved in H$_2$O$_2$ stress defense (70, 71). OxyR contains six cysteines, with Cys$^{199}$ and Cys$^{208}$ being highly conserved (4). The first step in OxyR’s activation occurs when Cys$^{199}$ is oxidized by peroxide to a sulfenic acid. The rate constant for this reaction is extremely high ($1.1 \times 10^5$ M$^{-1}$s$^{-1}$ to $\sim 10^7$ M$^{-1}$s$^{-1}$), ensuring activation of OxyR at H$_2$O$_2$ concentrations barely exceeding the physiological levels of H$_2$O$_2$ ($\sim 100$ nM) (4, 41, 72). However, activation of OxyR will be a competitive process with respect to other peroxide-detoxifying enzymes, such as catalases and peroxiredoxins, which react with H$_2$O$_2$ with equally high reaction rates. Sulfenic acid formation appears to induce an extrusion of Cys$^{199}$ from its hydrophobic pocket while introducing flexibility into the loop containing Cys$^{208}$, a residue that is 17 Å away from Cys$^{199}$ in the reduced form (Fig. 2B) (73). A second slower exchange leads to the formation of a disulfide bond between these two cysteines, locking OxyR in a transcriptionally active state (4, 73). Rotation of OxyR’s dimeric interface by 30° compared to the monomers in reduced OxyR changes the inter-dimer orientation of the tetramer, necessary for the cooperative binding of RNA polymerase and subsequent transcription of the target sequences (41, 73). The unusual stability of OxyR’s disulfide bond towards re-reduction by the Grx system allows gene transcription to proceed for extended periods of time despite a reducing redox environment (41, 72).

Since the discovery of OxyR’s redox-regulatory mechanism, numerous other pro- and eukaryotic transcriptional regulators have been identified that use reversible disulfide bond formation to mount effective responses against reactive oxygen and nitrogen species (Table S1). Their transcriptional activation is achieved by a variety of different means. Transcriptional repressors, such as the global regulator SarZ of *Staphylococcus aureus* or CprK of *Desulfitobacterium* spp. dissociate from DNA upon disulfide-mediated conformational changes, which causes de-repression of gene expression (74, 75). Oxidative disulfide bond formation in the transcriptional activator Yap1p leads to its nuclear accumulation, causing increased antioxidant gene expression (42). Similarly, transcriptional activation of FOXO/Daf16 is also controlled by nuclear translocation. Under oxidative stress conditions, an intermolecular disulfide bond between FOXO and the nuclear import factor Tnp1 forms, which appears to be crucial for the peroxide-mediated translocation of FOXO and the activation of antioxidant gene expression (76). This activation mechanism of FOXO is distinct from the insulin/P13K/PKD signaling pathway and represents an alternative mechanism of FOXO activation. Another strategy is employed by redox-regulated prokaryotic anti-sigma factors and eukaryotic inhibitors. Their disulfide-mediated degradation causes the release and activation of the respective transcriptional regulators, resulting in the activation of gene expression (77, 78).

**Redox-sensitive Active Site Cysteines as Functional Switches in Metabolic and Signaling Enzymes**

Active site cysteines are, by definition, highly reactive and hence prone to undergo oxidative modifications. One excellent example is the active site cysteine of glyceraldehyde-3-phosphate dehydrogenase (GapDH), which plays a crucial role in glycolysis. Upon exposure of GapDH to a variety of different ROS, sulfenic acid formation occurs at the active site cysteine, which appears to be responsible for the decrease in ATP levels observed in a variety of different species upon oxidative stress treatment (80, 81). Moreover, it contributes to the re-routing of glucose to the pentose phosphate pathway, generating NADPH instead of NADH (82). NADPH is a key factor in the reduction of the Trx and Grx system, and accordingly, increasing the
levels of NADPH raises the cell’s antioxidant potential. Note that oxidative modification of GapDH’s active site cysteines not only modulates its glycolytic activity but also leads to its nuclear translocation. In the nucleus, GapDH interacts with heterodimeric RNA and DNA-binding proteins, suggesting that GapDH may function as a transcriptional regulator during oxidative stress conditions (44).

The active site cysteine of protein tyrosine phosphatase (PTP) is another well-known target of oxidants, making tyrosine de-phosphorylation a redox-regulated signaling event in eukaryotes (83). While protein tyrosine kinases (PTK) are often activated under oxidative stress conditions (84), oxidation of PTPs generally leads to their inactivation. Under non-stress conditions, the active site thiolate of PTP (85) attacks the phosphorylated protein substrate, generating a cysteinyl phosphate intermediate, which is subsequently hydrolyzed (86). Exposure of PTP to oxidants, such as H₂O₂, leads initially to the formation of sulfenic acid, followed by the formation of reversible oxidation products, which differ among different PTPs. Disulfide formation with a nearby resolving cysteine appears to be the predominant oxidation product in the case of SHP1/2, PTEN, and Cdc25 (87-89) (Table S2). Oxidation inactivates the phosphatase activity, thus enhancing the signaling intensity achieved by substrate phosphorylation and therefore leading to the activation of signaling pathways, like NIK/IKK and MAPKs (ERK, p38, JNK). Their activation ultimately yields in the phosphorylation and activation of NFκB, causing expression of genes involved in antioxidant defense (84). Disulfide bond formation and other known oxidative PTP modifications, such as S-glutathionylation or sulfenamide formation, lead to conformational changes in PTP’s active site, which appear necessary for the subsequent re-reduction and reactivation of PTPs by members of the Trx or Grx system. This reduction terminates the signaling event.

**Peroxidases: Proteins of Many Traits**

Peroxiredoxins, a family of sulfhydryl-dependent peroxidases, are remarkable proteins with a variety of functions associated with oxidative stress defense. The primary function of peroxiredoxins appears to be the decomposition of H₂O₂. Driven by extremely high sensitivity and reactivity towards H₂O₂ (~10⁴ to ~2 × 10⁷ M⁻¹s⁻¹) (90, 91), peroxiredoxins rapidly undergo sulfenic acid formation at their active site cysteine. Subsequent disulfide bond formation with a nearby resolving cysteine and reduction of the disulfide bond by members of the thioredoxin system catalyzes the conversion of H₂O₂ to water (Fig. 3) (92).

Most typical reaction rates of cysteine thioles with H₂O₂ are extremely slow (~5–15 M⁻¹s⁻¹) (14, 17), which raises the intriguing question as to how cysteines in redox-regulated proteins other than peroxidases are being so rapidly oxidized by H₂O₂ in vivo (17, 90). Several studies now demonstrate that peroxidases have the capacity to perform catalytic thiol-disulfide exchange reactions, thereby influencing DNA transcription and cellular signaling (93-96). One well-studied example is the oxidative modification and activation of Yap1p by glutathione peroxidase 3 (Gpx3/Orp1 in yeast). Gpx3, whose active site cysteine is rapidly oxidized to sulfenic acid, reacts with a cysteine (Cys⁵⁹⁸) in Yap1p, forming a transient Gpx3- Yap1p intermolecular disulfide bond (97). The intermolecular disulfide bond is subsequently resolved by a thiol disulfide exchange reaction, which results in the reduction of Gpx3 and an intramolecular disulfide bond in Yap1p, leading to the activation of Yap1p (94, 97). A similar mechanism for oxidation equivalent transfer was reported for the oxidative activation of the apoptosis signaling kinase 1 (ASK1)/p38 pathway (93). In this case, oxidized peroxiredoxin 1 forms a disulfide exchange intermediate with ASK1. This leads to the oxidation and activation of ASK1 and the reduction of peroxiredoxin 1, making it ready for another round of peroxide detoxification (93, 96). It now remains to be seen how many proteins are oxidized by peroxidases and what criteria make them client proteins.

**Conclusion**

Reversible disulfide bond formation is a highly versatile post-translational mechanism employed by an ever-growing list of proteins to rapidly adjust their functional activity the moment reactive oxygen species start to accumulate. With the development of highly sensitive and quantitative redox proteomic techniques (5, 98, 99), we have now entered an era where it will be
feasible to determine the complete inventory of oxidation-sensitive protein thiols in organisms. This analysis will determine how redox-regulated checkpoints are embedded into a variety of metabolic pathways and how cells rapidly switch between distinct catabolic or anabolic processes, protect particularly vulnerable intermediates, and activate survival pathways in response to oxidative stress. The discovery of conserved redox-regulated pathways that improve oxidative stress tolerance in eukaryotes will be invaluable in understanding how proteins and pathways can be manipulated to decrease or increase their oxidative stress resistance, thereby facilitating the discovery of new treatment possibilities for diseases in which the redox balance is compromised.

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References


FIGURE LEGENDS

FIGURE 1. Reversible and irreversible cysteine modifications. Oxidation of cysteine thiol (R-SH/R-S-) by ROS, RNS, or RCS leads to the formation of highly reactive sulfenic acid (R-SOH), which can either react with another thiol to form a disulfide bond (R-S-S-R) or with glutathione (GSH) to become S-glutathionylated (R-S-S-G). These oxidative modifications are reversible and reduction is catalyzed by the thioredoxin (Trx) and/or glutaredoxin (Grx) system. Further oxidation of sulfenic acid to sulfinic acid (R-SO2H) and sulfonic acid (R-SO3H) is thought to be generally irreversible in vivo.

FIGURE 2. Redox-mediated activation of oxidative stress defense proteins. A, hypochlorous acid (HOCl)-mediated activation of Hsp33’s chaperone activity involves formation of two intramolecular disulfide bonds, zinc release (red sphere), and the unfolding of the C-terminal redox switch domain. Once activated, Hsp33 functions as an ATP-independent chaperone holdase, able to bind numerous unfolding client proteins to prevent their aggregation. When oxidative stress conditions subside, Hsp33’s disulfide bonds are re-reduced by the thioredoxin (Trx) system, triggering the transfer of client proteins to the DnaK/J/GrpE system for refolding. B, peroxide (H2O2)-mediated activation of the transcriptional activator OxyR involves formation of an intramolecular disulfide bond between two cysteines, which are separated by 17Å in the reduced form. These extensive conformational changes allow binding of RNA polymerase and transcription of target genes. The oxidative modifications are reversed by the Grx system, whose expression is under OxyR control.

FIGURE 3. The versatile life of peroxiredoxins. Peroxiredoxins (red) catalyze the decomposition of H2O2, transiently forming a sulfenic acid in this process. Disulfide bond formation and subsequent reduction by Trx regenerates the active site cysteine thiol in peroxiredoxin (A). In selected eukaryotic peroxiredoxins, high levels of H2O2 lead to the overoxidation of sulfenic acid to sulfinic acid, causing inactivation of the peroxidase function and concomitant activation as a chaperone holdase (B). ATP-dependent sulfiredoxins convert the sulfinic acid back to sulfenic acid, which is subsequently reduced by Trx. Peroxiredoxins also undergo disulfide-thiol exchange reactions, leading to the oxidative activation of selected proteins. After oxidation of their active site cysteine to sulfenic acid, an intermolecular disulfide bond forms between peroxiredoxin (e.g., Orp1) and the client protein (e.g., Yap1p) (green). This disulfide bond is subsequently resolved, leading to the formation of an intramolecular disulfide bond in the client protein and the release of reduced peroxiredoxin (C).
Figure 1

- Thiol
- Sulfenic acid
- Sulfenic acid oxidation
- Sulfinic acid
- Sulfonic acid

Reactions involved:

- ROS, RNS, RCS
- Trx
- GSH
- Grx
- Disulfide bond
- S-glutathionylation
Figure 2

A

inactive Hsp33 \rightarrow \text{HOCl} \rightarrow \text{Zn}^{2+} \rightarrow \text{active Hsp33}

\text{TrxA + DnaK/J, GrpE}

B

inactive OxyR \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{SOH SH} \rightarrow \text{S-S} \rightarrow \text{active OxyR}

\text{Grx system}