**The Redox Biochemistry of Protein Sulfenylation and Sulfinylation**

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Controlled generation of reactive oxygen species (ROS) orchestrates numerous physiological signaling events (1). A major cellular target of ROS is the thiol side-chain (RSH) of cysteine (Cys), which may assume a wide range of oxidation states (i.e., -2 to +4). Within this context, Cys sulfenic (Cys-SOH) and sulfinic (Cys-SO2H) acids have emerged as important mechanisms for regulation of protein function. Although this area has been under investigation for over a decade, the scope and the biological role of sulfenic / sulfinic acid modifications have been recently expanded with the introduction of new tools for the monitoring of cysteine oxidation in vitro and directly in cells. This review discusses selected recent examples of protein sulfenylation and sulfinylation from the literature, highlighting the role of these post-translational modifications (PTMs) in cell signaling.

**SULFENIC ACID FORMATION AND REACTIVITY**

RSOH is directly generated by the oxidation of RSH with two-electron oxidants (Figure 1A). Hydrogen peroxide (H2O2) reacts with small-molecule thiols at a constant rate of around 20 M$^{-1}$ s$^{-1}$, but this reaction can take place up to eight orders of magnitude faster (10$^5$ - 10$^8$ M$^{-1}$ s$^{-1}$) with specific Cys residues within proteins (2). The propensity of Cys residues to undergo oxidation is mainly influenced by three general factors: thiol nucleophilicity, surrounding protein microenvironment, and proximity of the target thiol to the ROS source. Peroxide-mediated thiol oxidation is an SN$_2$ reaction (Figure 1B) whereby the actual reactive species is the much more nucleophilic thiolate (RS$^-$). Accordingly, susceptibility to oxidation is usually correlated with pK$_a$, although for cysteines having pK$_a$<7, the RS$^-$ becomes less nucleophilic with the decrease of pK$_a$ value (3). In proteins, microenvironments can influence Cys acidity through the presence of polar amino acids or specific hydrogen bonds, which contribute to a decrease in pK$_a$ by balancing the negative charge on the sulfur atom (4). The same interactions, which affect the pK$_a$ of Cys thiol, also influence the stability of the related sulfenic acid. The microenvironment can also help to stabilize the leaving group by lowering the transition-state energy barrier (2). However, these parameters are not sufficient to rationalize the selective oxidation of specific proteins. Increasing evidence shows that ROS signaling responses are compartmentalized, and the proximity of the target protein to the ROS source is a key aspect of spatial regulation of Cys oxidation (5,6).

By virtue of the transient nature of RSOH, the study of its chemical-physical properties has been rendered extremely challenging. The pK$_a$ of RSOH has been determined in only a few proteins (7,8). The experimentally determined pK$_a$ of some small-molecule sulfenic acids is one/two orders of magnitude lower than the corresponding thiols (9,10); however, it is not clear whether such compounds are appropriate models of cysteine sulfenic acid in proteins. From the chemical point of view, RSOH exhibits both electrophilic and nucleophilic behavior. Thiosulfinate formation clearly exemplifies this dual nature (11), although this self-condensation has little biological relevance due to high abundant thiols and steric hindrance which make this reaction negligible in cells. Therefore, oxidation to Cys-SO2H appears to be the only significant reaction in which RSOH
exhibits its nucleophilic nature. On the other hand, this species shows high reactivity toward nucleophiles. Intramolecular or low-molecular weight thiols may react with RSOH to generate a mixed disulfide, which constitutes the principal mechanism for disulfide bond formation in proteins (12). In the absence of adjacent thiols, RSOH can also react with nitrogen nucleophiles to form a sulfenamide, though this species has been identified in only a few proteins (13,14).

**Sulfenic Acid as a Post-Translational Modification**

Disulfide and sulfenamide formations protect Cys-SOH from further oxidation and lay the foundation for redox signaling. In fact, these PTMs can generate conformational changes in protein structure and subsequent modulation of protein activity. In addition, as a result of its intrinsic nucleophilicity, Cys is present in the active site of many enzymes. Transient oxidation of these Cys residues is a well-established process through which proteins can be spatially and temporally inhibited.

From the first evidence of its existence reported in 1976 (15) to the present, RSOH has been identified in a relatively small number of proteins. In fact, the identification of this elusive modification remains difficult. In 2008, Fetrow et al. published a review that included a list of 47 proteins in which Cys-SOH was identified by crystal-structure analysis (16). Because identification of the crystal structure of protein-SOH is problematic, Fetrow et al.’s list represents only the tip of the iceberg. Direct mass analysis shows similar issues, making the use of chemical probes the only suitable technique to monitor RSOH formation (17).

**Table 1** provides a list of the principal proteins in which Cys-SOH has been identified using chemical-trapping reagents. In addition to NBD-Cl (4-Chloro-7-nitrobenzofurazan), which can be employed only *in vitro*, dimedone-based probes (DBPs) are emerging as the most promising tool for RSOH trapping. These reagents are capable of crossing the cellular membrane, capturing RSOHs directly in the cell (17). We concentrated our attention exclusively on those proteins where the formation of Cys-SOH has been experimentally substantiated and shown to play a regulatory role. Proteomic studies, based on the employment of DBPs, have identified many other proteins that are able to generate an RSOH-transient species in cells (18,19), although the biological relevance of theseoxidations remains unclear. **Table 1** clearly demonstrates that many redox-regulated proteins are directly involved in cell signaling.

**Protein Tyrosine Phosphatases**

Tyrosine phosphorylation levels are maintained by the balanced action of protein tyrosine kinases (PTKs) and phosphatases (PTPs). The sulfenylation of the PTPs’ catalytic Cys (pKₐ ranges from 4 to 6) has emerged as a dynamic mechanism for inactivation of this protein family (20). The half-life of RSOH is generally quite low in PTPs. In fact, a neighboring cysteine residue (e.g., in PTEN) or the backbone amide nitrogen (PTP1B) readily reacts with RSOH to yield, respectively, an intramolecular disulfide (21) or a cyclic sulfenamide species (14).

Recently, an alternative mechanism of inactivation emerged in SH2 domain-containing PTPs (SHP-1 and SHP-2) (22), which possess two highly conserved distal cysteines, both of which can generate a disulfide with the oxidized catalytic Cys. This intermediate disulfide typically rearranges into the more stable sulfenamide formed by the two backdoor cysteines to regenerate the free catalytic Cys residue. Surprisingly, the conformational change produced by the -backdoor disulfide leads to an increased catalytic Cys pKₐ value (~9) with resultant inhibition.

Although SHP-1 and SHP-2 have structural similarities, they are regulated by different cell-signaling pathways. SHP-2, for example, exhibits selective oxidation in response to platelet-derived growth factor (PDGF) in association with a PDGF receptor (23). However, these regulatory differences can be influenced by the method used to analyze their oxidation state. Employing an indirect RSOH detection method, T-cell activation, which induces H₂O₂ production, has shown transient oxidation of SHP-2 but not of SHP-1 (24). In a later work in which a DBP was used, both SHP-1 and SHP-2 showed Cys oxidation after T-cell activation, although with a different response time (25). These results could be rationalized by the greater sensitivity of direct RSOH analysis.

In *vitro* experiments have demonstrated that H₂O₂ deactivates SHP-1 and SHP-2 with second-
order rate constants of 2.0 M$^{-1}$ s$^{-1}$ and 2.4 M$^{-1}$ s$^{-1}$ respectively (22). These values are similar to those observed with other PTPs and are apparently too low to justify their oxidation within the cellular context. Recently, our group has observed that, following epidermal growth factor (EGF) stimulation, SHP-2 forms a complex with the EGF receptor (EGFR) and Nox2 (6), which could provide an explanation to its highly propensity to oxidation. In a similar manner, PTP1B, which is localized exclusively on the cytoplasmic face of the endoplasmic reticulum (ER), appears to be oxidized through the H$_2$O$_2$ generated by Nox4, an NADPH oxidase highly abundant in the ER (5). These two examples highlight the importance of the proximity of the target protein to the ROS source in explaining PTP oxidation.

Kinases

Increasing research has highlighted the key role of H$_2$O$_2$ in the modulation of PTKs activity. In comparison with PTPs, which are always inhibited by ROS, the oxidation of PTKs can lead to both enhancement and inhibition of kinase activity (26, 27).

The central role of Cys oxidation in PTK activity is exemplified by the redox control of EGFR signaling. EGFR is a receptor tyrosine kinase (RTK) activation of which is involved in the regulation of cellular proliferation, differentiation, and survival. In addition to promoting the tyrosine phosphorylation of protein targets, EGFR stimulation triggers the production of endogenous H$_2$O$_2$ by Nox activation. This localized increase in H$_2$O$_2$-concentration leads to the sulfenylation of a conserved Cys residue located within the intracellular kinase domain of EGFR (Cys797), which enhances its tyrosine kinase activity (6). The redox regulation of EGFR could represent a more general mechanism for the modulation of other RTK activity. In fact, nine additional members of this family show a Cys structurally analogous to EGFR Cys797, although further studies are needed in this direction.

Recently, Akt (a serine/threonine protein kinase) was also identified as a redox target. PDGF stimulation of fibroblasts induced H$_2$O$_2$ production, which led to isoform-specific regulation of Akt2 (28). A cysteine (Cys124), positioned in the linker region connecting the pleckstrin homology (PH) domain to the kinase domain, was found to be susceptible to sulfenylolation. Cys124-SOH can generate a disulfide with two distinct Cys residues – Cys297 and Cys311 – located in the kinase domain. This modification negatively modulates Akt2, although in vitro experiments showed that sulfenylolation formation has no direct effect on kinase activity. The inhibition mechanism remains unclear, but a previous work showed that Akt oxidation enhances its association with protein phosphatase 2A (PP2A), which could promote dephosphorylation of Akt (29).

Transcription Factors

In addition to the redox switch of PTKs and PTPs activities, which indirectly regulate transcription factors (TFs), H$_2$O$_2$ can directly modulate several TFs through the formation of intra- and intermolecular disulfide bonds (30). The first evidence of a redox-sensitive TF was identified in OxyR, a bacterial transcription factor, in which Cys-SOH mediated disulfide bond formation between Cys199 and Cys208 (31). Many other TFs are redox-regulated in prokaryotes (32-36), but relatively few cases have been identified in eukaryotes. In yeast, the activation of Yap1 through the formation of Gpx3-SOH mediates the oxidation of Yap1 through the formation of Gpx3-Yap1 intermolecular disulfide (37,38).

The anti-apoptotic NF-kB remains the only mammalian TF in which formation of Cys-SOH has been verified experimentally; however, this modification may also have a role in other peroxide-sensitive pathways of gene activation, such as the Nrf2/Keap1 system (39). H$_2$O$_2$ negatively switches NF-kB's DNA affinity – directly through the oxidation of its p50 subunit at Cys62 (40) and indirectly via Cys179 sulfenylation of the $\beta$ subunit of the IKK complex (IKK$\beta$), the kinase that is responsible for the NF-kB activation along the canonical pathway (41).

Cysteine Proteases

Protein ubiquitination has emerged as a central PTM whereby lysine residues are conjugated to ubiquitin (Ub), a 76 amino acid polypeptide (42). Deubiquitinating enzymes (DUBs) cleave ubiquitin or ubiquitin-like proteins from the target,
contributing to the balance of the Ub system. Four of the five different families of DUBs are cysteine proteases, which share in common a low-\(pK_a\) Cys residue essential for the catalytic mechanism.

Recently, three distinct works have shown that Cys oxidation can modulate DUB activity. Cotto-Rios et al. reported transient sulfenylation of catalytic Cys for several members of the Ub-specific protease (USP) family and for UCH-L1 (43). In particular, the authors were able to establish that USP-1, a DUB involved in DNA damage-response pathways, is reversibly inactivated following the induction of oxidative stress in cells. Additionally, Komander, in collaboration with our group, demonstrate that many members of the ovarian tumor (OUT) DUBs also undergo Cys oxidation upon H\(_2\)O\(_2\) treatment (44), including the tumor suppressor A20. Crystal-structure analysis of oxidized A20 showed that transient RSOH can be stabilized by the formation of hydrogen bonds with the highly conserved residues located in the loop preceding catalytic Cys. Both works noted that each DUB member exhibits a distinct level of sensitivity to oxidation. Differences in behavior can reflect various ranges of catalytic activation in which the conformational inactive enzyme could be less susceptible to oxidation. Lee et al. confirmed this hypothesis by showing that pre-incubation of USP7 with ubiquitin, which behaves as an allosteric activator, increased USP7 sensitivity to ROS (45).

An analogous inhibition has been found in small Ub-like modifier (SUMO) proteases. H\(_2\)O\(_2\) treatment induces RSOH-mediated formation of an intermolecular disulfide in the yeast SUMO protease Ulp1 as well as in its human equivalent, SENP1 (46). Interestingly, SUMOylation also appears to be redox-regulated by reversible oxidation of the catalytic Cys of SUMO conjugating enzymes (47), although no clear evidence of Cys-SOH formation has been provided.

### Ion Channels

It is well established that ROS plays a regulatory role for some ion channels (48), but little is known about the molecular mechanism through which this modulation is explicated. For example, human T-helper lymphocyte ORAI1 channels, a family member of Ca\(^{2+}\) release-activated Ca\(^{2+}\) (CRAC) channels, are inhibited by oxidation of the extracellular Cys195 (49), although the nature of this Cys oxidation remains unknown.

One exception is represented by the redox-regulation of Kv1.5, a potassium voltage-gated channel expressed in the heart and in pulmonary vasculature. Several studies have highlighted the fact that increased ROS concentration in cells is correlated to a reduction in Kv1.5 expression but have not provided a clear relationship between the two events. In collaboration with Martes’ laboratory, we were recently able to elucidate the specific mechanism for Kv1.5 channel redox-regulation (50). Labeling studies with DBPs have shown that a single Cys residue, located in the extracellular C-terminal domain of Kv1.5 (Cys581), forms a Cys-SOH after H\(_2\)O\(_2\) exposure. This modification triggers channel internalization, blocking its recycling to the cell membrane and promotes Kv1.5 degradation.

### Cellular Lifetime of Sulfenic Acid

Although limited solvent access and nearby hydrogen bond acceptors would contribute to RSOH stabilization, the absence of proximal thiols capable of generating an intramolecular disulfide is considered a major stabilizing factor.

In the absence of neighboring Cys residues, RSOH can be directly reduced to RSH by Trx (Figure 2A - Cycle 1) or may react with GSH to generate a mixed disulfide, which is later reduced by glutaredoxin (Figure 2A - Cycle 2). For example, human serum albumin (HSA) has only one free cysteine (Cys34), which is susceptible to H\(_2\)O\(_2\) oxidation (rate constant 2.5 M\(^{-1}\) s\(^{-1}\)). We can estimate the half-life of HSA-SOH based on its reaction with GSH. Using the known second-order rate constant for this reaction (~3 M\(^{-1}\) s\(^{-1}\)) (51) and estimating GSH concentration at 1 mM, the first-order rate constant would be 0.003 s\(^{-1}\). Substituting this value in the equation \(t_{1/2} = \ln2/k\), the estimated half-life of HSA-SOH would be ~4 minutes.

On the other hand, many redox-regulated proteins have a second proximal Cys that can form an internal disulfide with RSOH (Figure 2B). In Cdc25c, for example, Cys377-SOH reacts with “backdoor” Cys330 at a rate constant of 0.012 s\(^{-1}\) (52). Applying the same calculations as above, the half-life of Cdc25c-SOH would be ~1 minute. Taken together, these estimated protein-SOHs half-lives correlate well to the sulfenylation
papers published by our group and they appear
similar to the cellular lifetimes of many other
PTMs such as phosphorylation. In A431 cells, we
observed a peak of protein sulfonylation around 5
minutes after EGF stimulation, with a subsequent
decay over 30 minutes (6).

SULFINIC ACID FORMATION AND
REACTIVITY

RSOH may be over-oxidized to RSO$_2$H by
two-electron oxidants (Figure 1A). This reaction
requires nucleophilic attack by RSOH on the
peroxide species. Although the H$_2$O$_2$-mediated
oxidation of RSOH can proceed through two
possible pathways (Figure 1C), the pH profile
indicates that sulfenate anion (RSO$^-$) is the
reacting species. Therefore, the pK$_a$ value of
RSOH should influence this reaction (7). As we
emphasized above, the formation of a more stable
disulfide (or sulfenamide) should prevent RSOH
oxidation. Taking Cdc25c as an example, the
oxidation of Cys377-SOH to RSO$_2$H has a rate
constant of 110 M$^{-1}$ s$^{-1}$ (52); this value is on par
with the general tendency of protein-SOHs to
oxidation, which is generally in the range of 10-
10$^3$M$^{-1}$ s$^{-1}$ (7,51,52). Since internal disulfide
formation has a rate constant of 0.012 s$^{-1}$, the
oxidation of Cys377-SOH has significance only
over 100 μM of H$_2$O$_2$.

With a pK$_a$ value of around 2, RSO$_2$H exists
exclusively in deprotonated form at physiological
pH. The sulfinate group (RSO$_2$), which behaves
primarily as a soft nucleophile (53), shows low
spontaneous reactivity in cells and, because it is
not reducible by typical cellular reductants, its
oxidation to sulfonic acid (RSO$_3$H - Figure 1A)
appears to be the only relevant reaction in cells.
The considerations adduced above for RSOH
stability can also be applied to RSO$_2$H; therefore,
the formation of hydrogen bonds and steric
hindrance may stabilize Cys-SO$_2$H within
proteins, reducing its propensity to oxidation (54).

Sulfinic Acid as a Post-Translational
Modification

Cys-SO$_2$H was long considered merely an
artifact of protein purification. However,
increasing evidence indicates that hyperoxidation
to RSO$_2$H is not a rare event. Indeed, quantitative
analysis of soluble proteins from rat liver has
shown that around 5% of Cys residues exist as
Cys-SO$_2$H (55). Finally, the discovery of
Sulfiredoxin (Srx), an ATP-dependent protein that
specifically reduces Cys-SO$_2$H in the
peroxiredoxin (Prx) family, has opened the door to
an additional layer of redox regulation and
increased interest in this specific modification
(56).

Table 2 provides a list of proteins in which a
biological functional role has emerged for RSO$_2$H.
In comparison with Table 1, the number of
reported proteins is decidedly exiguous. This does
not necessarily indicate that RSO$_2$H plays a
negligible role in protein redox-regulation but
rather reflects the lack of robust methods for
monitoring the formation of such modifications
within proteins. Although, RSO$_2$H shows higher
stability in comparison to RSOH, mass and
crystal-structure analyses can introduce a high
percentage of artifacts. In addition, the emerging
relevance of persulfide modification (RSSSH) –
which has the same nominal mass shift as 32 Da –
makes the use of high-resolution mass
spectroscopy essential (57). We believe that the
development of chemical probes capable of
specifically trapping RSO$_2$H will push this Cys
modification from the minor role to which it has
been relegated. In this connection, we recently
proposed the use of aryl-nitroso compounds as
chemoselective probes for RSO$_2$H (58).

Peroxiredoxins and Sulfiredoxin

Prxs are a family of cysteine-based peroxidases
that remove H$_2$O$_2$ and other peroxides from cells.
Being highly abundant and exceptionally efficient
(second constant rate of 10$^7$–10$^8$ M$^{-1}$ s$^{-1}$), Prxs
maintain the cytosolic concentration of H$_2$O$_2$
under 100 nM (59). Therefore, regulation of Prx
activity is required to trigger H$_2$O$_2$-mediated
intracellular signaling.

Typical Prxs exist in antiparallel dimeric or
decameric forms and possess two Cys residues:
“peroxidatic” Cys (C$_P$), which reacts directly with
H$_2$O$_2$ to generate Cys-SOH, and “resolving” Cys
(C$_R$), which forms an intramolecular disulfide with
transient sulfenic acid. Finally Trx reduces
disulfide, restoring the catalytic cycle. Eukaryotic
2-Cys Prxs possess two sequence motifs (GGLG
and YF) in their C-termini that reduce the ability
of C$_R$ to approach C$_P$-SOH (60). The resulting
decrease in the disulfide-formation rate allows a
second molecule of H$_2$O$_2$ to react with C$_p$-SOH (Figure 2C), generating a Cys-SO$_2$H. Such overoxidation leads to the deactivation of peroxidase activity and the formation of high-molecular-weight aggregates, which exhibit molecular chaperone activity (61). Although just 0.1% of the C$_p$ in human PrxI is oxidized to Cys-SO$_2$H during each turnover (62) at low concentrations of H$_2$O$_2$, recent kinetic studies demonstrate that Prxs 2 and 3 can undergo appreciable hyperoxidation without requiring recycling of the disulfide (63).

The peroxidase activity of 2-Cys Prxs is restored by Srx (64). The first step in the proposed catalytic mechanism involves the oxygen attack of RSO$_2^-$ on the γ-phosphate of ATP and the resulting generation of a sulfenic phosphoryl ester (Figure 2C). This species represents a sort of activated SO$_2$H, which collapses to a thiosulfinate intermediate (Prx-(O)-S-Srx) after attack by a conserved Cys residue in Srx (65). Thiosulfinate is subsequently resolved by a third reducing species. Kinetic studies show that Srx is an inefficient enzyme. The rate of Prx-SSO$_2$H reduction is indeed rather low ($k_2 > 120$ s$^{-1}$, $k_3 \sim 85$ s$^{-1}$), suggesting that Prx requires a slow reparation process in order to allow H$_2$O$_2$ transient accumulation in response to extracellular signals.

**Parkinson’s Disease Protein DJ-1**

DJ-1 is a homodimeric small protein that has been associated with early onset Parkinson’s Disease (66). Many studies demonstrate that DJ-1 protects cells against oxidative stress-mediated apoptosis; however the mechanism of its protective function remains largely unknown (54).

A conserved Cys residue, Cys106, is extremely sensitive to oxidation and tends to form a Cys-SO$_2$H species generation of which appears to be critical for DJ-1 function. The highly conserved Glu18 residue facilitates the ionization of Cys106, reduces its $pK_a$, and helps to stabilize Cys106-SO$_2$H through the formation of an unusually short and consequently strong hydrogen bond (67). Wilson et al. have shown that small changes in this position can drastically influence the oxidation propensity of Cys106. For example, in the E18D DJ-1 mutant, the distance between the thiolate and the protonated carboxylic side chain is increased and Cys106 is predominantly oxidized to sulfenic acid (68). In fact, Asp18 tends to stabilize Cys106-SOH, hampering further oxidation. On the contrary, the structurally similar E18N mutant shows increased propensity to oxidation even in the absence of H$_2$O$_2$. More important, E18D mutants fail to protect cells from ROS while E18N showed similar levels of cell viability in comparison to the wild type, demonstrating that Cys106 oxidation to RSO$_2$H is essential for maintaining protective functions (69).

Considering Cys106’s high propensity to oxidation, it has been proposed that DJ-1 acts merely as a direct ROS scavenger. However, an elegant new study reported that the C106DD DJ-1 mutant is still able to protect cells against oxidative stress (70), excluding direct scavenger action by Cys oxidation.

**Cysteine oxidation and metal binding properties**

Cys residues are very common in metal-binding motif and can form coordinative bonds with several metal ions, including zinc, copper, and iron. Many proteins contain a Cys-Zn-Cys complex, for example, which furnishes structural rigidity. Oxidation of these cysteines causes Zn$^{2+}$ release and a subsequent conformational change, which can switch protein function. Although oxidation is usually transient, through the formation of a disulfide bond, in some cases it can lead to irreversible Cys-SO$_2$H (71).

Redox zinc switching is also involved in the activation of matrix metalloproteinases (MMPs). Matrilysis (MMP-7) contains a highly conserved cysteine switch sequence, PRCGVPDVA, in its pro-domain. The thiolate side-chain coordinates the catalytic Zn$^{2+}$, contributing to the maintenance of enzyme inactivity. Fu et al. showed that hypochlorous acid (HOCI), but not H$_2$O$_2$, can activate the enzyme through the conversion of Cys residue to RSO$_2$H, which disrupts zinc-coordination (72). An analogous redox-mechanism also appears to be involved in the activation of other MMPs (73).

The unique active site of nitrile hydratase (NHase) offers a sort of compendium of thiol oxidation states and metal coordinations. Structural analysis reveals that NHase contains an Fe$^{III}$ or Co$^{III}$-active site, in which three Cys residues, having three different oxidation states (RSH, RSOH and RSO$_2$H), contribute to the coordination of the metal ion (74,75). The fully reduced enzyme appears inactive, suggesting that
Cys sulfenylation and sulfinylation are critical in maintaining the catalytic activity of NHase (76), probably by increasing the Lewis acidity of the metal ion. An analogous motif was more recently found in the catalytic site of thiocyanate hydrolase (SCNase), which incorporates Co$^{III}$ only after Cys oxidation (77).

The active site of NHase and SCNase suggests that the oxidation state may influence Cys-binding properties, switching the affinity from zinc (for RSH) to iron and cobalt (for oxygenated sulfur species). This change could provide additional redox control of protein functions (78).

CONCLUSIONS AND PERSPECTIVES

Protein sulfenylation influences a wide range of PTMs both directly and especially indirectly (through the switching of protein function). We have seen how the oxidation of specific Cys residues in PTPs, PTKs, and cysteine proteases may regulate levels of phosphorylation, ubiquitination, and SUMOylation in cells. The modulation of transcription factors, and channel activity by Cys-SOH adds another level to the redox-signaling cascade.

The role of protein sulfinylation in cell signaling appears mainly confined in the Prx/Srx pair. We believe that the development of specific chemical probes for RSO$_2$H may help to find new Srx substrates or alternative reducing systems. Generally speaking, there is an urgent need for new protocols to analyze the full proteome and identify new targets. A deeper exploration of Cys oxidation in relation to metal-binding properties could open up new vistas on redox signaling. Finally, the development of drugs that specifically target the oxidative state form of proteins would appear to be a worthwhile goal (79).
REFERENCES


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<td><em>V. cholerae</em></td>
<td>Cys235&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(35)</td>
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<tr>
<td></td>
<td>MgrA</td>
<td><em>S. aureus</em></td>
<td>Cys12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(32)</td>
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<tr>
<td></td>
<td>SarZ</td>
<td><em>S. aureus</em></td>
<td>Cys13&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>(36)</td>
</tr>
<tr>
<td></td>
<td>OhrR</td>
<td><em>B. subtilis</em></td>
<td>Cys15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(34)</td>
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<td></td>
<td>OxyR</td>
<td><em>E. coli</em></td>
<td>Cys199&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(88)</td>
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<td></td>
<td>CrfJ</td>
<td><em>R. capsulatus</em></td>
<td>Cys420&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(33)</td>
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<tr>
<td></td>
<td>p50 (NF-kB )</td>
<td><em>H. sapiens</em></td>
<td>Cys62&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Cysteine Protease</td>
<td>USP1</td>
<td><em>H. sapiens</em></td>
<td>Cys90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(43)</td>
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<td>USP7</td>
<td><em>H. sapiens</em></td>
<td>Cys223&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(43)</td>
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<td></td>
<td>A20</td>
<td><em>H. sapiens</em></td>
<td>Cys103&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Cathepsin K</td>
<td><em>H. sapiens</em></td>
<td>Cys25&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>Papain</td>
<td><em>P. latex</em></td>
<td>Cys25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(80)</td>
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<td>Channel</td>
<td>Kv1.5</td>
<td><em>H. sapiens</em></td>
<td>Cys581&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(50)</td>
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<tr>
<td>Oxidoreductase</td>
<td>GAPDH</td>
<td><em>O. cuniculus</em></td>
<td>Cys298&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(81)</td>
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<td>Aldose reductase</td>
<td><em>H. sapiens</em></td>
<td>Cys298&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>MsrA</td>
<td><em>S. cerevisiae</em></td>
<td>Cys72&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>(91)</td>
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<td>Transferase</td>
<td>MTAP</td>
<td><em>H. sapiens</em></td>
<td>Cys136 / Cys223&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(92)</td>
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<td>α7β1</td>
<td><em>R. rattus</em></td>
<td>Cys923 / Cys928&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Integron</td>
<td>Hsp70</td>
<td><em>H. sapiens</em></td>
<td>Cys306&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Serum Protein</td>
<td>HSA</td>
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<td>Cys34&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>Oxygen carrier</td>
<td>Hemoglobin</td>
<td><em>H. sapiens</em></td>
<td>Cys 93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(95)</td>
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<td>Apoptotic regulator</td>
<td>Bcl-2</td>
<td><em>H. sapiens</em></td>
<td>Cys158 / Cys229&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(96)</td>
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<td>Cytoskeleton Protein</td>
<td>β-actin</td>
<td><em>H. sapiens</em></td>
<td>Cys272&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(97)</td>
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<sup>a</sup> Identified using dimedone or dimedone-based probes; <sup>b</sup> Identified using NBD-Cl; <sup>c</sup> Identified by crystal structure.
<table>
<thead>
<tr>
<th>Function</th>
<th>Protein</th>
<th>Organism</th>
<th>Cys-SOH</th>
<th>References</th>
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<td>Peroxidase</td>
<td>Prx</td>
<td><em>H. sapiens</em></td>
<td>Cys51&lt;sup&gt;d&lt;/sup&gt; (PrxI)</td>
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<td>Chaperone (?)</td>
<td>DJ-1</td>
<td><em>H. sapiens</em></td>
<td>Cys106&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>YaiL</td>
<td><em>E. coli</em></td>
<td>Cys106&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(98)</td>
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<td>Oxidoreductase</td>
<td>D-Amino Acid Oxidase</td>
<td><em>T. variabilis</em></td>
<td>Cys108&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Protease</td>
<td>MMP-7</td>
<td><em>H. sapiens</em></td>
<td>Pro-domain Cys&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(72)</td>
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<td>Hydratase</td>
<td>NHase</td>
<td><em>P. thermophilia</em></td>
<td>Cys131&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>S-transferase</td>
<td>L-PGDS</td>
<td><em>H. sapiens</em></td>
<td>Cys65&lt;sup&gt;d&lt;/sup&gt;</td>
<td>(100)</td>
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<sup>c</sup> Identified by crystal structure; <sup>d</sup> Identified by mass.
Figure Legends

Figure 1. Main oxidative modifications of protein cysteine residues. (A) The diagram shows the main oxidative modifications of protein cysteine residues. The initial reaction of cysteine with oxidants, \([O] = \text{ROS} / \text{RNS}\), yields a sulfenic acid (SOH). Once formed, the SOH can be reduced to thiol or further oxidized to generate cysteines \(\text{SO}_2\text{H}\) and \(\text{SO}_3\text{H}\). (B) Thiolate anion is much more nucleophilic of the corresponding protonated form and can be readily oxidized to sulfenic acid. The protein microenvironment can help to stabilize the poor hydroxide-leaving group and thus, accelerate the reaction rate. (C) Two possible mechanisms have been proposed for the \(\text{H}_2\text{O}_2\)-mediated oxidation of RSOH to \(\text{RSO}_2\text{H}\): a first pathway, which involves the direct participation of a sulfenate anion, or a second concerted mechanism, which is mediated by a hydrogen bond.

Figure 2. Sulfenic and sulfinic acid redox cycles. (A) R-SOH can be directly reduced to free thiol by Trx, although the importance of this pathway in cells is still debated (Cycle 1). RSOH can also react with GSH to generate a mixed disulfide (although not all protein-SOHs are accessible to GSH), which is subsequently reduced by Grx (Cycle 2). (B) In the presence of a neighboring Cys, RSOH forms an internal disulfide that is later reduced by Trx (Cycle 3). (C) Typical eukaryotic 2-Cys Prx are inactivated by over-oxidation to sulfinic acid (Step 1). Srx restores the sulfenic acid group using an ATP-dependent mechanism in which an activated sulfinic phosphoryl ester is generated (Step 2). This intermediate collapses to form a thiosulfinate moiety with Cys99 of Srx (Step 3). It has been proposed that this intramolecular thiosulfinate is finally resolved by a common cellular reductant, such as GSH or Trx, with consequent release of Prx-SOH (Step 4).
Figure 2

A

\[ \begin{align*}
  H_2O_2 & \rightarrow R-S^- \quad k_1 \\
  R-S^- & \rightarrow R-SOH \quad k_2 \\
  R-SOH & \rightarrow R-SO_2H \quad k_3 \\
  R-SO_2H & \rightarrow R-S^{-} \quad k_4 \\
  \text{Grx} & \rightarrow \text{GSH} \quad k_5 \\
  \text{R-S-SG} & \rightarrow \text{Trx} \quad k_6
\end{align*} \]

B

\[ \begin{align*}
  H_2O_2 & \rightarrow R'_{-}SH \quad k_6 \\
  R'_{-}SH & \rightarrow R'_{-}SOH \quad k_7 \\
  R'_{-}SOH & \rightarrow R'_{-}SO_2H \quad k_8 \\
  R'_{-}SO_2H & \rightarrow R'_{-}S^{-} \quad k_9 \\
  \text{Trx} & \rightarrow \text{R-S-S} \quad k_{10}
\end{align*} \]

C

\[ \begin{align*}
  H_2O_2 & \rightarrow \text{Prx-SO}_2H \quad k_{11} \\
  \text{Prx-SO}_2H & \rightarrow \text{Srx} \quad k_{12} \\
  \text{Srx} & \rightarrow \text{ATP} \quad k_{13} \\
  \text{ATP} & \rightarrow \text{ADP} \quad k_{14} \\
  \text{ADP} & \rightarrow \text{Prx-S-O-PO}_4^{2-} \quad k_{15} \\
  \text{Prx-S-O-PO}_4^{2-} & \rightarrow \text{RSH} \quad k_{16} \\
  \text{RSH} & \rightarrow \text{Prx-S-S-Srx} \quad k_{17}
\end{align*} \]