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Multiple Functions of Peroxiredoxins: Peroxidases, Sensors and Regulators of the Intracellular Messenger H_2O_2, and Protein Chaperones

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Abstract

Peroxiredoxins (Prxs) are a family of peroxidases that reduce peroxides, with a conserved cysteine residue (the peroxidatic Cys) serving as the site of oxidation by peroxides. Peroxides oxidize the peroxidatic Cys–SH to Cys–SOH, which then reacts with another cysteine residue (typically the resolving Cys [C_R]) to form a disulfide that is subsequently reduced by an appropriate electron donor. On the basis of the location or absence of the C_R, Prxs are classified into 2-Cys, atypical 2-Cys, and 1-Cys Prx subfamilies. In addition to their peroxidase activity, members of the 2-Cys Prx subfamily appear to serve as peroxide sensors for other proteins and as molecular chaperones. During catalysis, the peroxidatic Cys–SOH of 2-Cys Prxs is occasionally further oxidized to Cys–SO_2H before disulfide formation, resulting in inactivation of peroxidase activity. This hyperoxidation, which is reversed by the ATP-dependent enzyme sulfiredoxin, modulates the sensor and chaperone functions of 2-Cys Prxs. The peroxidase activity of 2-Cys Prxs is extensively regulated *via* tyrosine and threonine phosphorylation, which allows modulation of the local concentration of the intracellular messenger H_2O_2. Finally, 2-Cys Prxs interact with a variety of proteins, with such interaction having been shown to modulate the function of the binding partners in a reciprocal manner. *Antioxid. Redox Signal.* 15, 781–794.

Introduction: From Thiol-Specific Antioxidant to Thioredoxin Peroxidase to Peroxiredoxin

While purifying glutamine synthetase from *Saccharomyces cerevisiae* in 1985, we noticed that the highly purified enzyme gradually lost activity and degraded when stored in a buffer containing dithiothreitol (DTT) or 2-mercaptoethanol (48). Similar inactivation occurred when the thiol compound was replaced with ascorbate. The damage could be prevented by removal of either oxygen or trace amounts of contaminating Fe^{3+} from the buffer. These observations led us to speculate that the damage was caused by hydroxyl radicals ($HO^{\bullet}$), which indiscriminately oxidize organic molecules. These radicals are generated as molecular oxygen is reduced by an electron donor such as a thiol or ascorbate to the superoxide anion ($O_2^{\bullet -}$) and H_2O_2 and the latter is further reduced to $HO^{\bullet}$ *via* the iron-catalyzed Fenton reaction.

The question naturally arose as to why glutamine synthetase in the crude yeast extract was not inactivated in the presence of similar buffers containing DTT and Fe^{3+}. One possible answer was that the crude extract contained a factor that conferred protection against inactivation by reactive oxygen species (ROS). By supplementing a thiol-containing buffer with Fe^{3+} at micromolar levels, we were able to increase the rate of glutamine synthetase inactivation such that a measurable extent occurred within several minutes. With the use of this inactivation assay, we purified a 27-kDa protein (later determined more accurately to be 25 kDa) from yeast extract as the factor responsible for protection of glutamine synthetase against oxidation in the presence of O_2, thiol, and Fe^{3+} (47). However, despite the fact that both the thiol- and ascorbate-based oxidation systems generate ROS ($O_2^{\bullet -}$, H_2O_2, and $HO^{\bullet}$), the protective activity of the 25-kDa protein was observed only when a thiol compound such as DTT, 2-mercaptoethanol, or glutathione (GSH) was included in the oxidation system as an electron donor; when the thiol was replaced with ascorbate, no protective activity was apparent. In addition, the 25-kDa protein did not exhibit detectable superoxide dismutase or catalase activity. We therefore initially thought that the antioxidant function of the 25-kDa protein was not attributable to the elimination of $O_2^{\bullet -}$ or H_2O_2 but rather to the elimination of reactive sulfur species such as $RS^{\bullet}$, $RSSR^{\bullet}$, or $RSOO^{\bullet}$, which were known to be produced by the thiol oxidation system consisting of O_2, thiol, and Fe^{3+} (46, 47). On the basis of this belief, we named the 25-kDa protein

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thiol-specific antioxidant (TSA). No other enzyme that eliminates reactive sulfur species was known to exist. As described below, our speculation proved incorrect and TSA turned out to be an inappropriate name.

Although the precise nature of the damaging species eliminated by the 25-kDa protein was not known at that time, we assumed that this protein functioned as an antioxidant enzyme given that exposure of yeast to oxidative stress (100% O_2) resulted in a large increase in the synthesis of the protein. An antioxidant role was further supported by the observation that the growth rate of a TSA deletion mutant of yeast was similar to that of the wild-type strain under anaerobic conditions but was substantially lower than that of the wild-type strain under aerobic conditions, especially in the presence of oxidative stress exerted by peroxides (46).

Subsequently, a rat brain protein with a size similar to that of the yeast antioxidant enzyme was purified on the basis of its ability to protect glutamine synthetase from the thiol mixed-function oxidation system (14). The yeast, rat, and human genes that encode the 25-kDa TSA proteins were also cloned and sequenced (13, 14, 59). Sequence alignment revealed that the yeast- and mammalian-predicted proteins shared 65% amino acid identity. The deduced amino acid sequences showed no substantial similarity to any known catalase, superoxide dismutase, or peroxidase enzymes, however, consistent with the observation that TSA did not possess catalytic activity characteristic of the conventional antioxidant enzymes that protect against ROS.

Additional analysis of the purified 25-kDa TSA protein revealed that it does not contain conventional redox centers such as metals, heme, flavin, or selenocysteine. It therefore did not resemble any antioxidant known at the time. Critical information needed for further characterization of TSA came from studies of a seemingly unrelated enzyme, alkyl hydroperoxide reductase (Ahp), which had been identified in *Salmonella typhimurium* and *Escherichia coli*. Ahp, a heterodimer of 21-kDa AhpC and 57-kDa AhpF, catalyzes the conversion of alkyl hydroperoxides to their corresponding alcohols at the expense of NAD(P)H (36). The proposed catalytic mechanism for Ahp involved reduction of the substrate peroxide by AhpC, with subsequent rereduction of AhpC by AhpF coupled to NAD(P)H oxidation. A database search revealed substantial sequence similarity between TSA and AhpC, although initially this similarity appeared to be restricted to a small NH_2-terminal portion of the proteins. We resequenced the AhpC gene, however, and similarity of the encoded protein to TSA became apparent over the entire sequences (14). Importantly, the new sequence revealed the presence of a cysteine residue in the COOH-terminal region that is surrounded by a sequence that is also highly conserved in the three TSA proteins from yeast, rat, and human.

A database search subsequently revealed several additional proteins from a variety of organisms that also showed sequence similarity to TSA and AhpC (14). None of these additional AhpC/TSA family members had been associated with known biochemical reactions or characterized with reference to antioxidant function. Alignment of the 23 sequences of the AhpC/TSA family members present in the database in 1994 revealed two highly conserved cysteine residues corresponding to Cys^{47} and Cys^{170} of yeast TSA (14). The NH_2-terminal cysteine was conserved in all family members and the COOH-terminal cysteine was present in all except six proteins, thus dividing the AhpC/TSA family into 2-Cys and 1-Cys subgroups. We investigated the role of the conserved cysteines by replacing those of yeast TSA with serine (11, 14, 15). Assay for antioxidant activity based on the protection of glutamine synthetase against the thiol oxidation system revealed that the $Cys^{47} \rightarrow$ Ser (C47S) mutant was completely ineffective (15). Further analysis by gel electrophoresis under nondenaturing conditions indicated that wild-type TSA exists as a dimer, whereas the two cysteine mutants, C47S and C170S, exist as monomers. This observation suggested that the dimerization of TSA requires disulfide linkage of Cys^{47} and Cys^{170}, resulting in a head-to-tail arrangement of the two subunits. The presence of the Cys^{47}–S–S–Cys^{170} linkage was directly demonstrated by isolation of dimeric tryptic peptides, one monomer of which contained Cys^{47} and the other contained Cys^{170} (15). TSA thus became the first known antioxidant enzyme in which two cysteine residues form the essential redox center. The antiparallel dimeric structure was confirmed by X-ray crystallographic analysis of several AhpC/TSA homologs (31).

We then found that bacterial AhpC was as effective as TSA in protecting glutamine synthetase from inactivation by the thiol oxidation system (14). On the basis of this result, together with the sequence similarity between AhpC and TSA, we hypothesized that TSA may also act on peroxides and that the reduction of the resulting disulfide may be achieved by an enzyme with a function similar to that of AhpF. These hypotheses proved to be correct. Subsequent experiments showed that TSA provided protection against the thiol oxidation system by reducing H_2O_2. The apparent thiol specificity, which gave rise to the name TSA, is attributable to the fact that the TSA disulfide (Cys^{47}–S–S–Cys^{170}) can be reduced by a thiol but not by ascorbate. We also purified two protein components, thioredoxin (Trx) and Trx reductase (TrxR), which together mediate the flow of electrons from NADPH to the oxidized form of TSA (11, 14). The Trx system (Trx, TrxR, NADPH) was found to be more effective than DTT as a hydrogen donor for TSA, on the basis of its ability to support the antioxidant activity of the 25-kDa protein. In addition, the combination of Trx and TrxR was the only electron carrier detectable in yeast extract when NADPH or NADH was used as the ultimate electron donor for oxidized TSA.

Evidence suggested that, in the presence of the Trx system, TSA reduces peroxides with Trx as the immediate hydrogen donor and protects glutamine synthetase against the ascorbate oxidation system by eliminating H_2O_2 (11). We therefore proposed to rename TSA as thioredoxin peroxidase to reflect the fact that Trx is the immediate electron donor, and we abbreviated the name to TPx by analogy with GPx (glutathione peroxidase).

Given that the immediate electron donors identified for peroxide reduction by AhpC/TSA homologs were Trx or Trx-related proteins such as AhpF (for AhpC) and tryparedoxin (for tryparedoxin peroxidase of *Crithidia fasciculata*) (37, 71), the designation TPx appeared meaningful until Fisher and colleagues [see accompanying review by Fisher (24)] showed that mammalian 1-Cys peroxidase, in which only the NH_2-terminal cysteine is conserved, relies on GSH rather than Trx as the immediate electron source. It was also found that H_2O_2 as well as other peroxides such as lipid hydroperoxides and peroxynitrite ($ONOO^-$) are substrates of AhpC/TSA family members (8, 77, 87). We therefore selected peroxiredoxin (Prx,

also abbreviated as Prdx) as a more appropriate name to represent the family of peroxidases in which cysteine is the primary site of oxidation during the reduction of peroxides. "Peroxi-" indicates the nature of the substrate reduced, whereas "-redoxin" is reminiscent of Trx and glutaredoxin (Grx), which also contain redox-sensitive cysteines that undergo an oxidation–reduction cycle during protein function.

Three Distinct Types of Mammalian Prx and Their Reaction Mechanisms

Although Prx (formerly TSA and TPx, now Prx) was identified initially in yeast, the identification of multiple isoforms and their characterization were achieved first in mammalian cells. A database search in the mid-1990s revealed several mammalian proteins (NKEF A, PAG, MSP23, OSF3, HBP23, NKEF B, Calpromotin, Torin, MER5, SP22, AOE372, TrxRANK; these names were given without reference to peroxidase function) with sequence similarity to Prx enzymes identified in bacteria, yeast, rat, and human [reviewed in ref. (87)]. These proteins could be classified into four groups, which later defined four Prx isoforms (Prx I to IV). Both Prx I (NKEF A, PAG, MSP23, OSF3, HBP23) (33, 35, 45, 82, 93) and Prx II (NKEF B, Calpromotin, Torin) (33, 93) proteins consist of 199 residues and reside in the cytosol. The 257-amino acid sequence of Prx III (MER5, SP22) (101, 111) includes a 62-residue NH_2-terminal mitochondrial targeting sequence. Prx IV (AOE372, TrxRANK) (39, 62) contains a typical NH_2-terminal signal sequence for secretion but was recently found to be localized predominantly in the endoplasmic reticulum (34).

Like TSA initially identified in yeast, Prx I to Prx IV possess conserved NH_2-terminal and COOH-terminal cysteine residues that are separated by 121 amino acids and they belong to the 2-Cys subfamily. The four isoforms of mammalian 2-Cys enzymes (Prx I to Prx IV) share >70% amino acid sequence identity, with the homology being especially marked in the regions surrounding the conserved NH_2- and COOH-terminal cysteines. The peroxidase reaction mechanism of 2-Cys Prx enzymes is shown in Figure 1A. As demonstrated first with yeast TSA, Prx I to Prx IV exist as homodimers. The conserved NH_2-terminal Cys–SH (Cys^{52} for mammalian Prx I) is selectively oxidized by H_2O_2 to Cys–SOH, which then reacts with the conserved COOH-terminal Cys–SH (Cys^{173} of Prx I) of the other subunit to form an intermolecular disulfide. The disulfide is subsequently specifically reduced by Trx (14, 87, 109). Mammalian cells also contain mitochondrion-specific Trx and TrxR, suggesting that Prx III together with the mitochondrial Trx (97) and TrxR (56) provides a primary line of defense against H_2O_2 produced by the mitochondrial respiratory chain. The NH_2-terminal conserved cysteine was later referred to as the peroxidatic Cys (C_P) to reflect its sensitivity to oxidation by peroxides, and the COOH-terminal conserved cysteine was designated the C_R (107).

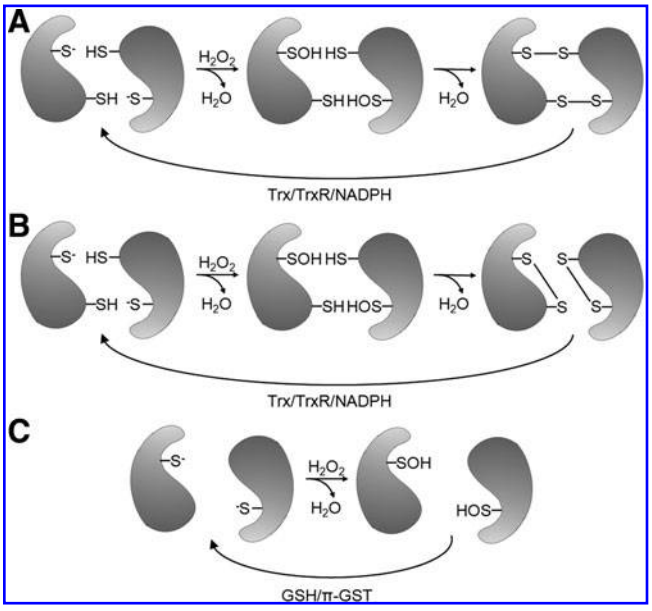

FIG. 1. Reaction mechanisms of peroxiredoxin (Prx) enzymes. Peroxidase reaction mechanisms of 2-Cys Prx **(A)**, atypical 2-Cys Prx **(B)**, and 1-Cys Prx **(C)** are shown.

A new Prx isoform, later named Prx V or atypical 2-Cys Prx, was identified independently as the result of characterization of a protein (AOEB166) in human bronchoalveolar lavage fluid (52) as well as of a human expressed sequence tag (EST) database search with the NH_2-terminal conserved sequence (KGKYVVLFFYPLDFTFVCP) of the 2-Cys Prx enzymes (92). Although the sequence surrounding the conserved NH_2-terminal cysteine (Cys^{47}) of Prx V is similar to that of 2-Cys Prx enzymes, the 162-amino acid Prx V shares only ~10% sequence identity overall with the four isoforms of mammalian 2-Cys Prx. The COOH-terminal region of Prx V is smaller than those of 2-Cys Prxs and lacks the conserved sequence containing the COOH-terminal cysteine of the latter enzymes. Prx V contains both mitochondrial (NH_2-terminal) and peroxisomal (COOH-terminal) sorting signals and is found in the cytosol as well as in mitochondria and peroxisomes (52).

Both human and mouse Prx V sequences contain cysteine residues at positions 72 and 151 in addition to the conserved Cys^{47}. However, the sequences surrounding Cys^{72} and Cys^{151} are not similar to those surrounding the COOH-terminal conserved cysteine of 2-Cys Prx enzymes, and the distances between Cys^{47} and the other two cysteines are substantially smaller than the 121 residues that separate the two conserved cysteine residues of typical 2-Cys Prxs. During the catalytic cycle of Prx V (Fig. 1B), the NH_2-terminal conserved Cys^{47}–SH (C_P–SH) is first oxidized by peroxides as in 2-Cys Prxs, and the resulting Cys^{47}–SOH then reacts with Cys^{151}–SH (C_R–SH) of the same subunit to form an intramolecular disulfide linkage, despite the fact that Prx V also exists in an antiparallel dimeric form (92, 95). The intramolecular disulfide formed by Prx V is reduced by Trx, but not by Grx or GSH. Although only the NH_2-terminal cysteine residue is conserved in Prx V, it is designated a 2-Cys Prx because its function is dependent on two cysteine residues. Unlike the mammalian Prx V, its plant homolog identified from poplar phloem was shown to accept reducing equivalents from both Trx and Grx (89). The structure, function, and regulation of Prx V are reviewed by Knoops *et al.* in this review series (53).

Prx VI, also termed 1-Cys Prx, was identified independently *via* several routes. A protein isolated initially as a nonselenium GPx in bovine eye (76, 77, 94) and rat olfactory mucosa (76, 77) was later shown to be a homolog of Prx (44) and termed Prx VI. We also recognized that the sequence of ORF06 obtained as the result of a random sequencing project

with human myeloid cell cDNA (68) was similar to the sequences of Prx enzymes and we demonstrated that the corresponding recombinant protein possessed DTT-dependent peroxidase activity (44). Interestingly, Prx VI was also identified on the basis of its phospholipase A_2 activity in rat lung (50). The peroxidase cycle of Prx VI is initiated when the NH_2-terminal conserved Cys–SH (C_P–SH), which corresponds to Cys^{47} of human Prx VI, is oxidized (Fig. 1C). However, the resulting Cys–SOH does not form a disulfide because of the unavailability of another Cys–SH nearby (19, 44). In addition to the cysteine corresponding to Cys^{47} of human Prx VI, some 1-Cys Prx enzymes contain other cysteine residues, such as Cys^{91} of the human enzyme. However, neither Cys^{91} of human Prx VI nor the sequence surrounding this residue is conserved among 1-Cys Prx enzymes (44). The C_P–SOH of oxidized Prx VI is reduced by nonphysiological thiols such as DTT but not by Trx or Grx (44). Conflicting reports as to whether GSH is capable of donating electrons to oxidized 1-Cys Prx were followed by the demonstration that the electron transfer to oxidized Prx VI requires its association with the π isoform of glutathione *S*-transferase (GST) (25, 61, 85), explaining the initial confusion regarding the physiological electron donor. The catalytic site for peroxidase activity of Prx VI is different from that for lipase activity (25). Prx VI, a bifunctional enzyme with peroxidase and lipase activities, is reviewed by Fisher in this series (24).

It is now evident that mammalian cells express six isoforms of Prx (I to VI) and that these six isoforms can be divided into three subgroups, namely 2-Cys, atypical 2-Cys, and 1-Cys. The common feature of Prxs is that the highly conserved peroxidatic Cys–SH (C_P–SH) is selectively and rapidly oxidized by the substrate peroxide. The first crystal structure obtained for a Prx enzyme was that of Prx VI, which revealed an antiparallel dimeric arrangement with two active sites where the thiolate anion (C_P–S^-) is stabilized by the positively charged guanido group of Arg^{132} (19). Subsequent determination of the structures of other types of Prx also revealed the interaction between C_P–S^- and this conserved arginine (2, 22, 31, 60, 80, 91). The conserved arginine, by stabilizing the thiolate anion, lowers the pK_a of the C_P–SH to between 5 and 6 from the corresponding value of 8.6 for the noninteracting residue (69, 72, 78). Given that the thiolate anion is more readily oxidized by peroxides than is its protonated thiol counterpart, the catalytic cysteine is selectively oxidized among the several cysteine residues present in the protein. The critical role of the conserved arginine located downstream of the peroxidatic cysteine was demonstrated by amino acid replacement (64). Structure-based explanations for the rapid reaction of Prx with peroxides and the characteristics of the various Prx enzymes are reviewed by Hall *et al.* in this series (29).

Although Prx VI is not at all similar to GPx enzymes in terms of amino acid sequence, Prx VI is a GSH-dependent peroxidase. Further, many GPx homologs contain a peroxidatic cysteine (C_P–SH) instead of selenocysteine in the active site and form a disulfide between the C_P–SH and another conserved cysteine (C_R–SH) on reaction with peroxides (60). As in 2-Cys Prx, the resulting disulfide is reduced by Trx or a Trx-like protein (60). The distinction between Prx and GPx families therefore becomes obscure. Another example for the dependence of Prx on GSH can be found from yeast Prx 1, which is a mitochondrial enzyme belonging to the 1-Cys Prx (74). Recent studies with *S. cerevisiae* Prx 1 (Prx1p) indicated that C_P–SOH of this enzyme is glutathionylated and then the resulting disulfide is reduced by TR coupled to GSH (28) or by the action of Grx and GSH (75). Similarities and dissimilarities between these families in terms of structure and peroxidase mechanism are also reviewed extensively by Flohe *et al.* in this series (26).

Hyperoxidation of 2-Cys Prx and the Role of Sulfiredoxin

While studying the kinetics of TPx1 (yeast 2-Cys Prx) and human Prx I, we observed that the rate of TPx1-dependent oxidation of NADPH monitored spectrophotometrically decreased gradually with time (113). This decay in activity was due to selective oxidation of C_P–SH to C_P–SO_2H. The C_P–SOH generated as an intermediate during catalysis thus occasionally undergoes further oxidation to C_P–SO_2H, a reaction that cannot be reversed by Trx. This hyperoxidation, which was also observed with purified mammalian 2-Cys Prx enzymes, appears to occur when disulfide formation between C_P–SOH and C_R–SH is slow enough to allow the reaction of C_P–SOH with peroxide. The distance between the sulfur atoms of C_P and C_R ($\sim$13 Å) in 2-Cys Prxs is large for rapid disulfide formation (31, 91). More importantly, eukaryotic 2-Cys Prxs harbor a conserved GGLG motif and a COOH-terminal extension containing a YF motif (108), and the interaction between these motifs is thought to impede the attack of the C_R–SH in the adjacent monomer of the dimer on the C_P–SOH to form a disulfide bond. The resulting catalytic "pause" permits excess peroxide to oxidize C_P–SOH further to C_P–SO_2H. A role for the COOH-terminal tail in this process was supported by the observation that yeast 2-Cys Prx lost its sensitivity to overoxidation on truncation of its COOH-terminal tail (54).

The presence of H_2O_2 alone is not sufficient to induce the hyperoxidation of Prx I. Rather, all the catalytic components (H_2O_2, Trx, TrxR, NADPH) are required, indicating that such hyperoxidation occurs only when Prx I is engaged in the catalytic cycle (113). After the disulfide has formed, the sulfur atom of C_P is thus resistant to further oxidation and becomes liable again to hyperoxidation only when it is in the sulfenic state. Kinetic analysis of mammalian Prx I inactivation in the presence of a low steady-state level (<1 μM) of H_2O_2 indicated that the enzyme was hyperoxidized at a rate of 0.072% per turnover at 30°C. Hyperoxidation of Prx I was also detected in various mammalian cells treated with H_2O_2 (113).

On investigation of the fate of hyperoxidized Prx molecules, we discovered that the C_P–SO_2H produced during exposure of cells to H_2O_2 is gradually reduced to the catalytically active thiol form (104). This discovery of the ability of mammalian cells to reduce protein sulfinic acid was surprising because it was generally believed that oxidation to the sulfinic state is an irreversible process in cells. The enzyme responsible for the reduction of sulfinylated Prx in yeast was subsequently identified (6). This enzyme, named sulfiredoxin (Srx), was initially identified on the basis of the marked upregulation of its expression by H_2O_2 and the reduced tolerance of cells to H_2O_2 conferred by deletion of its gene. Srx defines a conserved family of proteins in lower and higher eukaryotes, all of which share a conserved cysteine. Studies with the yeast and mammalian enzymes showed that reduction by Srx requires ATP hydrolysis, Mg^{2+}, and a thiol as an

electron donor (6, 17). Recent study indicated that sulfinic Prx was further oxidized to sulfonic form with C_P–SO_3H in yeast cells exposed to higher concentrations of H_2O_2 and that the sulfonic Prx could not be reduced by Srx (58). Mechanistic and structural studies revealed that Srx forms a quaternary complex with ATP, Mg^{2+}, and sulfinic Prx and that the reduction reaction proceeds through a sulfinic acid phosphoryl ester intermediate formed by the direct transfer of the γ-phosphate of ATP to Prx–SO_2H (Fig. 2) (41). Subsequently, the active site Cys-SH of Srx attacks the phosphoryl ester to produce a Srx-Prx thiosulfinate intermediate (42, 90), which is then resolved by RSH to generate active Prx-SOH (Fig. 2).

Oxidation of cysteine to sulfinic acid is not restricted to 2-Cys Prx enzymes. Critical cysteine residues of many other proteins including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), several glycolytic enzymes, carbonic anhydrase, metalloproteinases, the Parkinson's disease-associated protein DJ-1, and protein tyrosine phosphatases are also oxidized to sulfinic acid. Indeed, 1.4% of the cysteine residues of all soluble proteins in rat liver were detected as sulfinic acid (30). Atypical 2-Cys Prx (Prx V) and 1-Cys Prx (Prx VI) also undergo hyperoxidation, albeit more slowly than 2-Cys Prx. On examination of the susceptibility of sulfinic forms of various proteins to reduction by Srx, we found that the sulfinic forms of the four 2-Cys Prxs (Prx I to IV), but not those of Prx V, Prx VI, or GAPDH, were reduced *in vitro* (105). Further, mammalian Srx was found to bind specifically to the four 2-Cys Prxs both *in vitro* and in cells. Sulfinic forms of Prx I and Prx II, but not those of Prx VI or GAPDH, present in H_2O_2-treated A549 cells were gradually reduced after removal of H_2O_2; overexpression of human Srx increased the rate of reduction of Prx I and Prx II but did not affect that of Prx VI or GAPDH. These results suggest that reduction of Cys–SO_2H by mammalian Srx is specific to 2-Cys Prx isoforms (105). For proteins such as Prx VI and GAPDH, sulfinic acid formation might be an irreversible process that causes protein damage. The recently solved crystal structure of the Srx-Prx I complex revealed that the C-terminus of Prx I unfolded completely and packed onto the backside of Srx away from the catalytic site (40). This interaction might explain the substrate specificity of Srx.

Soon after the discovery of Srx, it was proposed that members of another family of proteins, designated sestrins (Sesns), possess reductase activity toward cysteine sulfinic acid of Prx (9). Sestrins are 48–60-kDa proteins that show no sequence similarity to Srx (13 kDa). The mechanism of the reduction of sulfinic 2-Cys Prx enzymes by Srx, the first known biological example of such a reaction, has been studied extensively by several laboratories. However, no similar effort has been described for sestrins, and a recent study showed that Sesn2 is not a reductase for cysteine sulfinic acid of Prx or other proteins (103).

Prokaryotic 2-Cys Prx enzymes, which do not contain the COOH-terminal GGLG and YF motifs of their eukaryotic counterparts, are insensitive to oxidative inactivation, and prokaryotes do not express Srx (6, 108, 109). Reversible inactivation through hyperoxidation has therefore been proposed to be a eukaryotic adaptation that allows H_2O_2 to accumulate to substantial levels under certain circumstances. Although all eukaryotes are equipped with a variety of enzymes to remove toxic H_2O_2 molecules, many mammalian cell types produce H_2O_2 in response to various extracellular stimuli and use this hazardous oxidant as a signaling molecule to mediate regulation of responses such as cell growth, cell differentiation, and gene expression (86). For example, stimulation of cells with platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) induces H_2O_2 production, and prevention of H_2O_2 accumulation results in inhibition of signaling by these growth factors (4, 23). For H_2O_2 to serve as a signal, its concentration must increase rapidly above a certain threshold and remain elevated long enough for it to react with effector molecules. Both Prx I and Prx II are abundant cytosolic proteins, together accounting for 0.5%–1% of total soluble protein in several types of cultured cells (12). The reversible inactivation of 2-Cys Prx *via* hyperoxidation has therefore been suggested to be the result of structural features acquired during evolution to accommodate the intracellular messenger function of H_2O_2 (108). The transient accumulation of H_2O_2 was proposed to be achieved through protection of the generated H_2O_2 molecules from destruction by Prx, a notion termed the "floodgate" hypothesis (108). Having found that the reduction of sulfinic forms of Prx I and Prx II by Srx is a slow process ($k_{cat} = 0.18\ min^{-1}$) (17), we also speculated that the slow rate of Prx reactivation *via* Srx-dependent reduction is a built-in mechanism that allows sufficient time for H_2O_2 to accumulate and propagate its signal. However, hyperoxidation of Prx I or Prx II was not observed in cells stimulated with various growth factors, indicating that growth factor signaling does not require inactivation of Prxs *via* hyperoxidation (20, 79, 106).

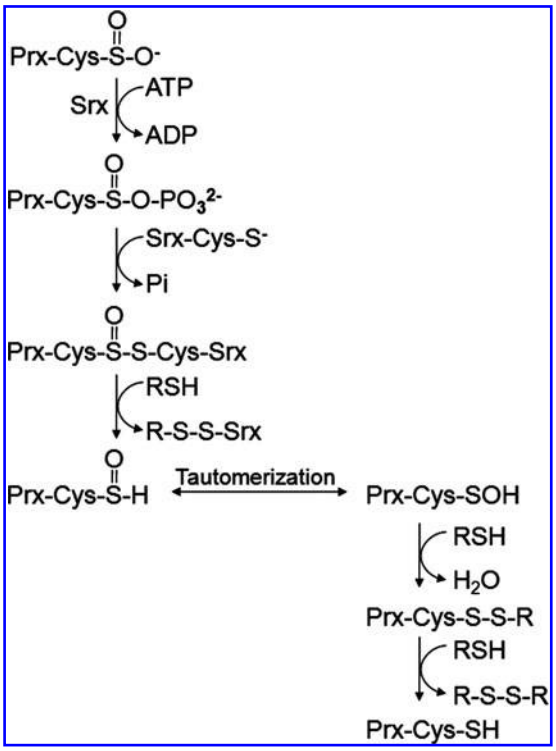

FIG. 2. The mechanism for the reduction of sulfinic 2-Cys Prx by sulfiredoxin (Srx). The direct attack of the Prx-Cys-SO_2^- on the γ-phosphate of ATP generates a sulfinic phosphoryl ester intermediate. And then, Cys^{99} in Srx attacks the intermediate to generate a Srx-Prx thiosulfinate intermediate that can be resolved by RSH to generate active Prx-SOH. RSH represents a thiol equivalent such as glutathione, dithiothreitol, or thioredoxin.

Physiological Roles of Hyperoxidized 2-Cys Prx

Hyperoxidation of 2-Cys Prxs results in a structural transition from a dimer to a toroid decamer that can undergo further aggregation, and the decameric and aggregated forms of the enzyme gain protein chaperone function (38, 65) (Fig. 3). This chaperone activity appears to correlate with the oligomerization state of the enzyme. The toroidal structures comprise five dimers linked together by hydrophobic interactions (63, 91, 109), with the redox state of the catalytic cysteine being a key factor determining the dimer–decamer equilibrium. The sulfinic enzymes thus favor the decameric form, whereas the disulfide enzymes exist predominantly as dimers. At the low concentrations of H_2O_2 present in cells under normal conditions, 2-Cys Prxs exist mostly as oligomeric structures of low molecular weight (dimers and decamers) that, in addition to removing low levels of H_2O_2, appear to protect proteins from denaturation. However, under conditions of oxidative stress, the 2-Cys Prxs rapidly undergo structural changes characterized by the conversion of the low-molecular-weight forms to complexes of high molecular weight (aggregates) that exhibit much higher chaperone activity (superchaperones activity) than dimers and decamers (38, 65) (Fig. 3). In the presence of Srx, which reduces C_P–SO_2H to C_P–SH, the dissociation of the high-molecular-weight complexes into low-molecular-weight species occurs on removal of H_2O_2. This change activates the peroxidase function of Prx and lowers the chaperone activity to the basal level (Fig. 3). These observations thus suggest that the reversible hyperoxidation of 2-Cys Prxs not only inactivates peroxidase activity but also greatly enhances the chaperone function of these enzymes.

Reversible Prx hyperoxidation is also thought to play a role in the regulation of transcription factors in yeast (7, 21, 99). In the yeast *Schizosaccharomyces pombe*, two independent but complementary signaling pathways are activated to induce distinct patterns of gene expression depending on the intracellular level of H_2O_2 (18, 84). The transcriptional response to low concentrations of H_2O_2 is dependent on the AP-1–like transcription factor Pap1, in which H_2O_2 promotes the formation of an intramolecular disulfide linkage between distant cysteine residues, Cys^{278} and either Cys^{501} or Cys^{532}. Oxidation of Pap1 results in the loss of its ability to interact with the nuclear exporter Crm1, leading to Pap1 accumulation in the nucleus and to Pap1-dependent gene expression (99, 100). The cysteine residues of Pap1 are not directly oxidized by H_2O_2; instead, TPx1 is oxidized to form a disulfide between C_P and C_R and then transfers the oxidation state to Pap1. Thus, TPx1, which serves as the upstream sensor of H_2O_2, is essential for the upregulation of Pap1-dependent transcriptional activity. At higher levels of H_2O_2, TPx1 is hyperoxidized to the sulfinic form and is thus no longer able to mediate disulfide formation in and thereby to activate Pap1. Instead, the mitogen-activated protein kinase (MAPK) Sty1 is activated by H_2O_2 and in turn phosphorylates and activates Atf1, a yeast homolog of the mammalian transcription factors Nrf2 and ATF2 (98). As the peroxide concentration increases, therefore, Pap1-dependent gene expression is downregulated, whereas Atf1-dependent transcription is upregulated. Srx is one of the gene products that are induced *via* the Sty1-Atf1 pathway. As a result of Srx induction, the catalytically inactive sulfinic TPx1 is reduced to the catalytically active form, which lowers the level of H_2O_2 and leads to activation of the Pap1 pathway. In support of this mechanism, overexpression of Srx in *Saccharomyces cerevisiae* was shown to reverse the peroxide-induced oxidation of TPx1 and to promote the activation of Pap1 at higher levels of H_2O_2 (7). Further, deletion of the Srx gene prevented reduction of hyperoxidized TPx1 and inhibited the activation of Pap1 and Pap1-dependent gene expression. The hyperoxidized or reduced status of TPx1 is thus the key determinant in the regulation of two independent transcription pathways by H_2O_2 signaling (21).

Reversible Inactivation of the Peroxidase Activity of Prx I and Prx II *via* Phosphorylation

Having found that the hyperoxidation of Prx I and Prx II is not the mechanism that allows the transient accumulation of

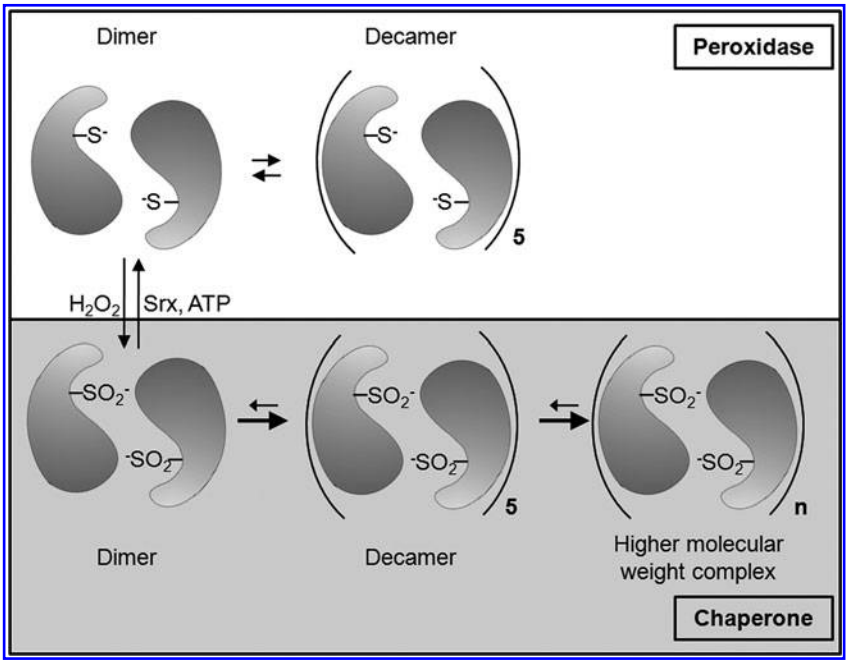

FIG. 3. Functional switching of 2-Cys Prx between a peroxidase and a chaperone. Members of the 2-Cys Prx subfamily exist in an equilibrium of dimers, decamers, and high-molecular-weight complexes. The equilibrium depends on the redox state of the catalytic cysteine, with hyperoxidation to the sulfinic enzyme favoring the high-molecular-weight complexes, which exhibit marked chaperone activity that is independent of peroxidase activity. Reduction of the sulfinic enzyme by Srx reactivates peroxidase activity and concomitantly suppresses chaperone activity by shifting the equilibrium in favor of dimers and decamers.

H_2O_2 for receptor signaling, we continued the search for such a mechanism, with our recent results implicating reversible inactivation of Prx I by tyrosine phosphorylation.

Prx I is phosphorylated on Tyr^{194}, mainly by tyrosine kinases of the Src family, in cells stimulated *via* receptors for growth factors such as PDGF or EGF or *via* immune receptors such as T-cell and B-cell receptors (106). Kinetic analysis revealed that phosphorylation of Prx I on Tyr^{194} conferred a marked latency to the onset of peroxidase activity and that the activity of the phosphorylated enzyme was lower than that of the nonphosphorylated enzyme. The absolute amount of Tyr^{194}-phosphorylated Prx I in stimulated cells was found to be low. For example, only 0.2%–0.4% of total Prx I was phosphorylated in NIH 3T3 cells stimulated with PDGF, the reason for which was shown to be that the phosphorylation reaction is confined to Prx I molecules associated with lipid rafts, which serve as a platform for the assembly of various signaling proteins (57). Such phosphorylation was thus not observed with Prx I present in the cytosol. Both growth factor and immune receptors are active within lipid rafts, and NADPH oxidase enzymes (Nox1 and Nox2) as well as Src family kinases are constitutively associated with these membrane microdomains. The spatially confined inactivation of Prx I thus provides a means for generating favorable H_2O_2 gradients around lipid rafts, where signaling proteins are concentrated, while minimizing the general accumulation of H_2O_2 to toxic levels and disturbance of global redox potential. These results indicate that localized production of H_2O_2 might not be sufficient to support the messenger function of this molecule in cells stimulated *via* growth factor or immune receptors, with inactivation of Prx I by phosphorylation on Tyr^{194} being necessary to protect the pool of H_2O_2 signaling molecules from destruction by this abundant and catalytically efficient enzyme (Fig. 4).

PDGF-induced hyperoxidation of Prx I or Prx II was not observed, indicating that H_2O_2 produced at lipid rafts by Nox does not hyperoxidize these Prx isoforms and that growth factor signaling does not require their inactivation at lipid rafts *via* hyperoxidation. Rather, hyperoxidation of Prx I or II appears to occur as the result of a global increase in the intracellular concentration of H_2O_2, which is consistent with the observation that Srx is induced only when cells are exposed to severe oxidative stress (3).

Human Prx I and Prx II share 76% sequence identity, and both proteins are localized in the cytosol and nucleus. Purified Prx II is phosphorylated by the nonreceptor tyrosine kinase c-Abl but at a much slower rate than that apparent for Prx I. However, tyrosine-phosphorylated Prx II was not detected in several cell types stimulated with EGF or PDGF, despite the fact that, like Prx I, Prx II was detected in lipid rafts. The difference in the rates of phosphorylation of Prx I and Prx II might be explained by the structures of these two enzymes (Fig. 5). Histidine-197 of Prx II appears to form a hydrogen bond with Asp^{181}, with this interaction possibly hindering access of a protein tyrosine kinase to Tyr^{193} (the residue corresponding to Tyr^{194} of Prx I). No analogous interaction is apparent in Prx I, in which Gln is present at the position corresponding to His^{197} of Prx II.

In addition, Prx I, but not Prx II, was found to undergo phosphorylation, whereas only Prx II underwent hyperoxidation, when cells were stimulated with H_2O_2 and PDGF at the same time. These results suggest that, unlike Prx I, Prx II is inactivated not *via* tyrosine phosphorylation but rather *via* hyperoxidation of its catalytic cysteine and only under conditions of sustained oxidative stress. This differential susceptibility of Prx I and Prx II to phosphorylation and hyperoxidation suggested that these enzymes might play distinct roles in cell signaling.

The peroxidase activity of Prx II *in vitro* is only 30% of that of Prx I (12), and it is extinguished more rapidly during catalysis as a result of a greater susceptibility of the C_P–SH to hyperoxidation. Differential hyperoxidation of Prx I and Prx II was examined in HeLa cells exposed to glucose oxidase in the presence of glucose to maintain a steady flux of low levels of H_2O_2 (106). Exposure of the cells to the generated H_2O_2 for 30 or 90 min resulted in the hyperoxidation of ~30% and ~60% of Prx II, respectively, whereas the corresponding values for Prx I were only ~5% and ~10%. Preferential hyperoxidation of Prx

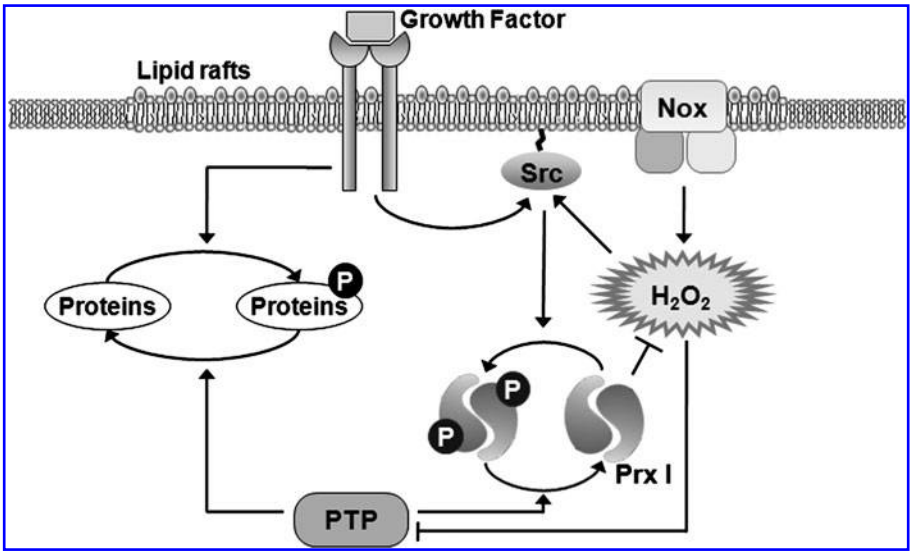

FIG. 4. Model for the mechanism underlying H_2O_2 accumulation around lipid rafts and its role in intracellular signaling. Engagement of a growth factor receptor induces the production of H_2O_2 through activation of NAPDH oxidase present in a lipid raft. The activated receptor also induces phosphorylation of raft-associated Prx I on Tyr^{194} by activating an Src family kinase, resulting in inactivation of Prx I, which would otherwise destroy the newly generated H_2O_2. Inactivation of Prx I allows the accumulation of H_2O_2 around lipid rafts, which in turn promotes further phosphorylation and inactivation of Prx I by both activating Src kinases and inactivating protein tyrosine phosphatases (PTPs). These two positive feedback loops allow the sustained H_2O_2 signaling necessary for the regulation of biological responses.

II was also observed in NIH 3T3 cells treated with paraquat (an H_2O_2 generator): Approximately 50% of Prx II was hyperoxidized, whereas Prx I hyperoxidation was not detected.

It thus appears that Prx I and Prx II are regulated differently: Prx I is selectively phosphorylated by protein tyrosine kinases and functions as a modulator of local H_2O_2 levels, whereas Prx II is more susceptible to hyperoxidation in cells subjected to sustained global oxidative stress. Although both Prx I and Prx II are associated with lipid rafts, inactivation of Prx I, which is more efficient as a peroxidase than is Prx II, might be sufficient to allow local accumulation of H_2O_2 for signal propagation. The role of raft-associated Prx II remains unclear. Given that the accumulation of a small amount of H_2O_2 at lipid rafts can trigger rapid signal amplification through the action of positive feedback loops, the presence of Prx II in addition to Prx I might function as a safeguard to prevent inappropriate activation of signaling cascades by basal production of H_2O_2 at rafts. Consistent with this notion, cells derived from Prx II-deficient mice exhibit enhanced tyrosine phosphorylation of the PDGF receptor when stimulated with PDGF compared with cells from wild-type animals (20). Functional differences between Prx I and Prx II are also apparent in the phenotypes of the corresponding knockout mice: Mice lacking Prx I thus manifest an increased susceptibility to cancer, whereas those deficient in Prx II do not (70). Prx I has thus been suggested to function as a tumor suppressor, a suggestion that was further supported by the observation that Prx I deficiency in mice increases the susceptibility to Ras- or ErbB2-induced breast cancer (10). Tumor suppression by Prx I has been attributed to its ability to bind to phosphatase and tensin homolog (PTEN) and to protect the lipid phosphatase activity of this protein from oxidative inactivation by removing H_2O_2 produced in response to proliferative signals. Prx II does not bind to PTEN. Given that Prx I activity is critical for maintaining the activation of protein tyrosine phosphatases and likely that of PTEN also in the vicinity of lipid rafts, tumor suppression by Prx I is also probably related to its ability to remove H_2O_2 from the region surrounding these microdomains.

Prx I and Prx II are also regulated through phosphorylation by cyclin-dependent kinases (Cdks) (16). Both Prx I and Prx II contain a consensus sequence (Thr-Pro-Lys-Lys, corresponding to residues 90–93 of Prx I and 89–92 of Prx II) for phosphorylation by Cdks and are phosphorylated specifically at this Thr residue by the cyclin B-dependent kinase Cdk1. Phosphorylation of Prx I at Thr^{90} results in inactivation of peroxidase activity. Experiments with HeLa cells arrested at various stages of the cell cycle showed that Prx I phosphorylation at Thr^{90} is apparent during mitotic phase but not in interphase and that it occurs in parallel with the activation of Cdk1. The phosphorylation of Prx I at Thr^{90} both *in vivo* and *in vitro* was inhibited by roscovitine, an inhibitor of Cdks. Further, depletion of Cdk1 by RNA interference prevented Prx I phosphorylation in cells synchronized in mitotic phase. Prx II is also phosphorylated by Cdk1 *in vitro*, albeit to a lesser extent than is Prx I. Cdk1-dependent phosphorylation and inactivation of Prx thus likely play a role in mitosis.

In addition to Prx enzymes, most cells contain catalase and GPxs for the elimination of H_2O_2. Given that catalase is localized in the peroxisome, H_2O_2 molecules would have to diffuse into peroxisomes before they could be acted upon by catalase. In addition, catalase is an inefficient scavenger at lower concentrations of H_2O_2 because the completion of its catalytic cycle requires two consecutive collisions with H_2O_2. However, given that some GPxs (especially GPx1) are in the cytosol and have the same kinetic mechanism of Prx I and Prx II, inactivation of Prx I and Prx II may not result in an increase in the intracellular concentration of H_2O_2. As in the case of Prx I phosphorylated at Tyr^{194} that allows transient accumulation of H_2O_2 around lipid rafts, inactivation of Prx I and Prx II *via* Thr phosphorylation might provide a means for generating favorable H_2O_2 gradients around a subcompartment of cytosol during mitosis. The relevance of a temporary increase in H_2O_2 levels during mitosis is not clear. Potential target molecules on which H_2O_2 might act to propagate its signal include dual phosphatases such as Cdc25C and Cdc14B (96). Cdc25C is an important regulator of Cdk1, and Cdc14B is a key regulator of the level of Polo-

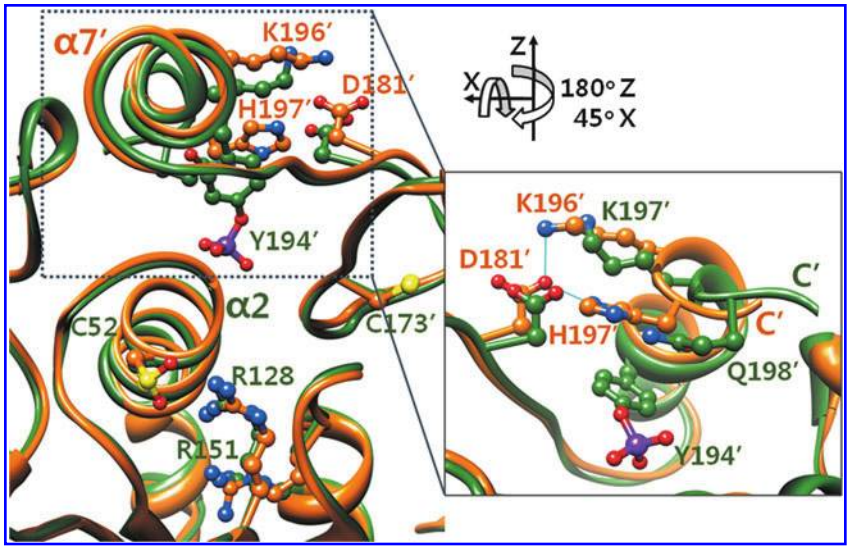

FIG. 5. Ribbon diagrams comparing the active sites and COOH-terminal regions of Prx I (green) and Prx II (gold). Histidine-197 of Prx II appears to form a salt bridge network with Asp^{181} and Lys^{196} in the same subunit, and this interaction appears to hinder access of a protein tyrosine kinase to Tyr^{193} (99). The salt bridge network of Prx II might stabilize the structure of the COOH-terminal domain, in which the salt bridge distance between Asp^{181} and Lys^{196} and that between Asp^{181} and His^{197} are 2.64 and 2.69 Å, respectively. Asp^{182} and Lys^{197} are conserved in Prx I, but Prx I contains Gln^{198} instead of His^{197} of Prx II. The salt bridge network is displayed in cyan in the enlarged image (*right*), which was obtained after a rotation of 180° around the *z*-axis followed by a rotation of 45° around the *x*-axis for the image on the *upper left*. Primed residue numbers correspond to the subunit whose catalytic Cys is not shown.

like kinase 1 (5). Like other protein tyrosine phosphatases, Cdc25 and Cdc14 enzymes contain an essential cysteine residue in a His-Cys-X_5-Arg motif (1) that is sensitive to oxidation by H_2O_2.

Another example of Prx regulation by threonine phosphorylation is observed in a model of Parkinson's disease (83). Cdk5 is hyperactivated and plays a major functional role in the loss of dopaminergic neurons in the mouse model of this disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a toxin for mitochondrial complex 1. Prx II directly associates with the Cdk5-p35 complex. In addition, Cdk5 phosphorylates Prx II at Thr^{89}, resulting in a reduction in peroxidase activity and neuronal death, in toxin-treated cells *in vitro* and *in vivo*. A mutant (T89E) of Prx II that mimics the constitutively phosphorylated enzyme did not protect neurons from the mitochondrial insult, whereas the wild-type protein or a mutant lacking the Thr^{89} phosphorylation site effectively promoted neuronal survival. Prx II phosphorylation was shown to be dependent on the Cdk5 complex by the observation that p35-deficient mice, which are resistant to death induced by the mitochondrial toxin, manifest a reduced level of Prx II phosphorylation and increased Prx II peroxidase activity. Moreover, p35-deficient neurons show reduced levels of ROS and improved survival. These findings provide a mechanistic link for how a mitochondrial toxin, through calpain-mediated activation of Cdk5 and consequent downregulation of the activity of an important antioxidant enzyme, can increase oxidative load, leading ultimately to cell death.

Prx-Binding Proteins

Various Prx-binding proteins have been identified by coimmunoprecipitation analysis or the yeast two-hybrid assay. The number of binding proteins for Prx I has been found to be greater that that for Prx II, and these Prx I-binding partners contribute to a variety of cellular functions. Although their precise roles have not been extensively characterized, Prx I and II appear to serve as sensors of H_2O_2 to regulate the function of their binding partners in a redox-dependent manner, as exemplified by the interaction of TPx1 and Pap1 in yeast. In other cases, the function of the binding protein appears to be regulated by Prx independently of the redox state of Prx. Modulation of the peroxidase activity of Prx by binding proteins has also been demonstrated. As shown by the association of Prx I and Prx II with lipid rafts, Prx isoforms can be localized to specific cellular compartments. Some Prx-binding proteins might thus serve as anchors for Prx compartmentalization. Prx-binding proteins identified to date include the following:

- Androgen receptor: Prx I directly interacts with the androgen receptor and enhances its transactivation of target genes in response to hypoxia-reoxygenation in prostate cancer cells (73).
- Apoptosis signal-regulating kinase 1 (ASK1): Under resting conditions, a small amount of hemagglutin epitope-tagged ASK1 coimmunoprecipitated with Myc epitope-tagged Prx I from transfected cells (49). Stimulation of the cells with H_2O_2, however, resulted in marked enhancement of the ASK1–Prx I interaction. Prx I interacts with ASK1 through its NH_2-terminal Trx-binding domain, and both Cys^{52} and Cys^{173} of Prx I are required for the interaction with ASK1 induced by H_2O_2. Prx I negatively regulates ASK1 signaling that results in activation of the p38 MAPK signaling pathway in response to H_2O_2.
- c-Abl: Prx I was identified as a protein that binds to the Src homology 3 domain of c-Abl by yeast two-hybrid screening and was shown to interact with c-Abl *in vivo* (102). Prx I inhibits c-Abl–dependent tyrosine phosphorylation in intact cells as well as the kinase activity of c-Abl *in vitro*. It also inhibited the cytostatic and cytotoxic effects of c-Abl.
- Cdk5-p35: Prx II was identified as a Cdk5-interacting protein in mouse brain extracts by a GST pull-down assay and was shown to associate with the Cdk5-p35 complex *in vivo* (83). Cdk5 phosphorylates Prx II at Thr^{89}, resulting in inhibition of Prx II peroxidase activity and in the death of neurons exposed to the mitochondrial toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine *in vitro* or *in vivo*.
- c-Myc: Prx I was shown to interact with the Myc box II domain of c-Myc by yeast two-hybrid screening (67). Prx I inhibits anchorage-independent growth of c-Myc–overexpressing fibroblasts and downregulates the expression of certain c-Myc target genes.
- Cyclophilin A: Cyclophilin A was identified as a binding protein for Prx VI in a crude extract of rat lung with an overlay assay (55). Cyclophilin A enhanced the antioxidant activity of Prx VI as well as that of the other mammalian Prx isotypes and was able to serve as a general electron donor for all mammalian Prxs. Both Cys^{115} and Cys^{161} of human cyclophilin A were found to be required for the activation and reduction of Prx.
- eEF1A-2: Prx I was found to bind the mouse translation elongation factor eEF1A-2 by yeast two-hybrid screening (73). Prx I coimmunoprecipitated with eEF1A-2, but not with its isoform eEF1A-1, from extracts of mouse tissues. Cotransfection with vectors for both Prx I and eEF1A-2 protected cells from peroxide-induced apoptosis.
- Glycerophosphodiester phosphodiesterase 2 (GDE2): Prx I was identified as a protein that interacts with GDE2 by large-scale immunoprecipitation of FLAG-tagged GDE2 from extracts of embryonic chick spinal cord (112). Prx I synergizes with GDE2 to promote spinal neuron differentiation. It binds and activates GDE2 by reducing an intramolecular disulfide bond between the NH_2- and COOH-terminal domains of GDE2 that normally gates its function.
- GSTπ-c-Jun NH_2-terminal kinase (JNK) complex: Prx I associates with the complex of GSTπ and the MAPK JNK and thereby suppresses the ionizing radiation-induced release of JNK from this complex and JNK activation (51). Expression of a Prx I mutant that lacks peroxidase activity suppressed the dissociation of JNK from the complex as effectively as that by wild-type Prx I in irradiated cells.
- Macrophage migration inhibitory factor (MIF): Prx I was identified as a MIF-interacting protein by yeast two-hybrid screening (43). Prx I binds specifically to MIF *in vivo*, and this interaction appears to be dependent on redox status because it was inhibited under reducing conditions. Binding of Prx I to MIF suppresses the D-dopachrome tautomerase activity of MIF. Moreover,

this binding resulted in inhibition of the peroxidase activity of Prx I.

- Omi/HtrA2: Omi/HtrA2, a member of the large HtrA serine protease–chaperone family, was identified as a human Prx I-interacting protein by yeast two-hybrid screening (32). The COOH-terminal region of Prx I specifically interacts with the PDZ domain of mature Omi/HtrA2, resulting in an increase in protease activity.
- p66Shc: Prx I was identified as an interaction partner for the NH_2-terminal region (p66CH2CB) of p66Shc by pull-down experiments (27). This interaction resulted in disassembly of the decameric form of Prx I as well as in reduction of the p66CH2CB tetramer, which impaired its ability to induce mitochondrial rupture. These results indicate that p66CH2CB and Prx I form a stress-sensing complex that maintains p66Shc inactive as long as stress levels remain moderate.
- PDGF receptor: Prx II associates with PDGF receptor-β on PDGF stimulation and suppresses inactivation of protein tyrosine phosphatases (20). Prx II deficiency results in increased production of H_2O_2, enhanced activation of the PDGF receptor and phospholipase C–γ1, and increased cell proliferation and migration in cells stimulated with PDGF. Prx II also suppresses PDGF receptor activation in endothelial cells and thereby inhibits vascular remodeling in a murine model of restenosis.
- Phospholipase D1 (PLD1): Interaction of Prx II with PLD1 was identified with a PLD1-affinity pull-down assay (110). Phorbol 12-myristate 13-acetate stimulation promoted the physical interaction between PLD1 and Prx II in cultured cells, and overexpression of Prx II specifically inhibited the activation of PLD1 in cells stimulated with H_2O_2.
- Presenilin-1: Prx I was identified as a presenilin-1–interacting protein by yeast two-hybrid screening (115). In addition, Prx I and presenilin-1 were coimmunoprecipitated from both transfected cells and mouse brain extract. Microinjection of a plasmid encoding Prx I into sympathetic neurons from the superior cervical ganglion in primary culture resulted in neuronal apoptosis. However, coinjection of a plasmid for wild-type presenilin-1 with that for Prx I prevented Prx I-induced apoptosis.
- Stomatin: Stomatin was found to interact with Prx II on affinity purification of the latter from human erythrocyte membranes (66). Prx II contributes to activation of Ca^{2+}-dependent K^+ transport in red cells (81), and stomatin is thought to play a regulatory role in monovalent cation transport.
- Syk: Prx I was identified by quantitative proteomics screening for proteins that bind to phosphorylated peptides of the protein tyrosine kinase Syk (114). Prx I preferentially bound to a Syk peptide containing phosphorylated Tyr^{346}.
- Toll-like receptor 4 (TLR4): Prx I interacts with TLR4 and thereby enhances TLR4-dependent secretion of tumor necrosis factor-α and interleukin-6 from macrophages and dendritic cells (88). Prx I interaction with TLR4 occurs independently of its peroxidase activity and appears to be dependent on its chaperone activity and ability to form decamers. These findings suggest that Prx I may act as a danger signal similar to other TLR4-binding chaperone molecules such as HSP72.

Conclusion

2-Cys Prx enzymes are homodimeric and contain two conserved (peroxidatic and resolving) cysteine residues, which are the active site of peroxidase reaction. A specific positive environment around the peroxidatic cysteine provided by the antiparallel dimeric structure makes the members of 2-Cys Prx subfamily highly reactive toward peroxides, thus allowing them function as an effective peroxidase despite the lack of conventional redox centers such as heme, metals, and selenocysteine. The cysteine sulfenic acid resulting from the oxidation of peroxidatic cysteine by peroxides reacts with the C_R of the other subunit to form an intersubunit disulfide.

2-Cys Prx are more than just simple peroxidase-eliminating enzymes. 2-Cys Prx proteins interact with various proteins and serve as upstream sensors of H_2O_2 to regulate the function of their binding partners in a redox-dependent manner. The sensor function of 2-Cys proteins is attributed to their ability to react with very low levels of peroxides and to a major conformational change induced by disulfide formation between two distantly located peroxidatic and C_R residues.

During the catalysis of peroxidase reaction, the sulfenic form of the peroxidative cysteine is occasionally further oxidized to sulfinic acid, resulting in the inactivation of its peroxidase activity. The hyperoxidation results in the major structural change from low-molecular-weight oligomers to high-molecular-weight complexes, which accompanies a functional change from peroxidase to molecular chaperone. The chaperone activity can protect a protein substrate from thermal-induced aggregation resembling the function of heat shock proteins, which can also form well-ordered oligomers. The hyperoxidation can be reversed through an ATP-dependent process catalyzed by Srx.

Many mammalian cell types produce H_2O_2 in response to various extracellular stimuli and use this hazardous oxidant as a signaling molecule. Temporary protection of those intentionally produced H_2O_2 molecules from destruction by 2-Cys members, which are abundant in the cytosol, is achieved through tyrosine phosphorylation-dependent inactivation of peroxidase activity. Reversible inactivation of 2-Cys Prx enzymes *via* threonine phosphorylation appears to play a role in cell cycle progress.

Acknowledgments

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Abbreviations Used

Ahp = alkyl hydroperoxide reductase
ASK1 = apoptosis signal-regulating kinase 1
Cdk = cyclin-dependent kinase
C_R = resolving Cys
DTT = dithiothreitol
EGF = epidermal growth factor
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
GDE2 = glycerophosphodiester phosphodiesterase 2
GPx = glutathione peroxidase
Grx = glutaredoxin
GSH = glutathione
GST = glutathione *S*-transferase
$HO^{\bullet}$ = hydroxyl radicals
JNK = c-Jun NH_2-terminal kinase
MAPK = mitogen-activated protein kinase
MIF = macrophage migration inhibitory factor
$O_2^{\bullet -}$ = superoxide anion
PDGF = platelet-derived growth factor
PLD1 = phospholipase D1
Prx = peroxiredoxin
PTEN = phosphatase and tensin homolog
ROS = reactive oxygen species
Srx = sulfiredoxin
TLR4 = Toll-like receptor 4
TPx = thioredoxin peroxidase
Trx = thioredoxin
TrxR = thioredoxin reductase
TSA = thiol-specific antioxidant

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