Structure, mechanism and regulation of peroxiredoxins

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Peroxiredoxins (Prxs) are a ubiquitous family of antioxidant enzymes that also control cytokine-induced peroxide levels which mediate signal transduction in mammalian cells. Prxs can be regulated by changes to phosphorylation, redox and possibly oligomerization states. Prxs are divided into three classes: typical 2-Cys Prxs; atypical 2-Cys Prxs; and 1-Cys Prxs. All Prxs share the same basic catalytic mechanism, in which an active-site cysteine (the peroxidatic cysteine) is oxidized to a sulfenic acid by the peroxide substrate. The recycling of the sulfenic acid back to a thiol is what distinguishes the three enzyme classes. Using crystal structures, a detailed catalytic cycle has been derived for typical 2-Cys Prxs, including a model for the redox-regulated oligomeric state proposed to control enzyme activity.

Peroxiredoxins (Prxs) [1,2] have received considerable attention in recent years as a new and expanding family of thiol-specific antioxidant proteins, also termed the thioredoxin peroxidases and alkyl-hydroperoxide-reductase-C22 proteins. Prxs exert their protective antioxidant role in cells through their peroxidase activity ($ROOH + 2e^- \rightarrow ROH + H_2O$), whereby hydrogen peroxide, peroxynitrite and a wide range of organic hydroperoxides (ROOH) are reduced and detoxified [3–7]. Indeed, these enzymes are truly ubiquitous having been identified in yeast, plant and animal cells, including both protozoan and helminth parasites, and most, if not all, eubacteria and archaea.

Although located primarily in the cytosol, Prxs are also found within mitochondria, chloroplasts and peroxisomes, associated with nuclei and membranes, and, in at least one case, exported [3,8]. Prxs are produced at high levels in cells: they are among the ten most abundant proteins in *Escherichia coli* [9], the second or third most abundant protein in erythrocytes [10] and compose 0.1–0.8% of the soluble protein in other mammalian cells [11]. Many organisms produce more than one isoform of Prx, including at least six Prxs identified in mammalian cells (PrxI–PrxVI; Table 1). Recently, a range of other cellular roles have also been ascribed to mammalian Prx family members, including the modulation of cytokine-induced hydrogen peroxide levels, which have been shown to mediate signaling cascades leading to cell proliferation, differentiation and apoptosis [3,8,12,13]. The peroxidatic functions of Prxs probably overlap to some extent with those of the better known glutathione peroxidases and catalases, although it has been suggested that their moderate catalytic efficiencies ($\sim 10^5\ M^{-1}\ s^{-1}$) compared with those of glutathione peroxidases ($\sim 10^8\ M^{-1}\ s^{-1}$) [3] and catalases ($\sim 10^6\ M^{-1}\ s^{-1}$) [14] makes their importance as peroxidases questionable [3]. Nonetheless, the high abundance of Prxs in a wide range of cells and a recent finding that a bacterial Prx [alkyl hydroperoxide reductase C22 (AhpC)] and not catalase is responsible for reduction of endogenously generated H_2O_2 [15] argue that Prxs are indeed important players in peroxide detoxification in cells.

Prxs use redox-active cysteines to reduce peroxides and were originally divided into two categories, the 1-Cys and 2-Cys Prxs, based on the number of cysteinyl residues directly involved in catalysis [1]. Structural and mechanistic data now support the further division of the 2-Cys Prxs into two classes called the 'typical' and 'atypical' 2-Cys Prxs. The peroxidase reaction is composed of two steps centered around a redox-active cysteine called the peroxidatic cysteine. Based on existing data [16,17], all three Prx classes appear to have the first step in common, in which the peroxidatic cysteine ($Cys–S_PH$) attacks the peroxide substrate and is oxidized to a cysteine sulfenic acid (Cys–SOH) (Fig. 1) [16,18]. The peroxide decomposition probably requires a base to deprotonate the peroxidatic cysteine as well as an acid to protonate the poor RO^- leaving group, but these catalysts have yet to be identified. All Prxs to date conserve an active-site Arg, which would lower the pK_a of the peroxidatic cysteine somewhat by stabilizing its thiolate form (Fig. 1). The second step of the peroxidase reaction, the resolution of the cysteine sulfenic acid, distinguishes the three Prx classes.

The typical 2-Cys Prxs are the largest class of Prxs and are identified by the conservation of their two redox-active cysteines, the peroxidatic cysteine (generally near residue 50) and the resolving cysteine (near residue 170) [3]. Typical 2-Cys Prxs are obligate homodimers containing two identical active sites [19–22]. In the second step of the peroxidase reaction, the peroxidatic cysteine sulfenic acid ($Cys–S_POH$) from one subunit is attacked by the resolving cysteine ($Cys–S_RH$) located in the C terminus of the other subunit (Fig. 1). This condensation reaction results in the formation of a stable intersubunit disulfide bond, which is then reduced by one of several cell-specific disulfide

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Table 1. Six subclasses of Peroxiredoxins (Prxs) from mammals

Prx subtype	PrxI (2-Cys)	PrxII (2-Cys)	PrxIII (2-Cys)	PrxIV (2-Cys)	PrxV (atypical 2-Cys)	PrxVI (1-Cys)
Previous nomenclature	TPx-A NKEF A MSP23 OSF-3 HBP23 PAG	TPx-B NKEF B PRP Calpromotin Torin Band-8 TSA	AOP-1 SP22 MER5	AOE372 TRANK	AOEB166 PMP20 AOPP	ORF06 LTW4 AOP2
Polypeptide length	199 aa	198 aa	256 aa (cleaved at 63–64)[a]	271 aa (cleaved at 36–37)[a]	214 aa (cleaved at 52–53)[a]	224 aa
Human chromosomal location	1q34.1	13q12	10q25–q26	10p22.13	11q13	1q23.3
Cellular location	Cytosol, nucleus	Cytosol, membrane	Mitochondria	Cytosol, Golgi, secreted	Mitochondria, peroxisome, cytosol	Cytosol
Genbank SwissProt	AAA50464 tdx2_human P35703	AAA50465 tdx1_human P32119	BAA08389 tdxm_human P30048	AAB95175 tdxn_human Q13162	AAF03750 aopp_human P30044	BAA03496 aop2_human P30041
Interactions with proteins and other ligands	c-Abl Presenilin-1 Heme Macrophage migration inhibitory factor Cyclophilin	Protein 7.2b (stomatin) Presenilin-1 Erythrocyte membrane Cyclophilin	Cyclophilin Abrin A-chain	Heparin Cyclophilin	DNA Cyclophilin	Cyclophilin

Abbreviation: aa, amino acids.
[a]These proteins are post-translationally processed.

oxidoreductases (e.g. thioredoxin, AhpF, tryparedoxin or AhpD [23–25]), completing the catalytic cycle.

The second class of Prxs are the atypical 2-Cys Prxs, which have the same mechanism as typical 2-Cys Prxs but are functionally monomeric [26,27]. In these Prxs, both the peroxidatic cysteine and its corresponding resolving cysteine are contained within the same polypeptide, with the condensation reaction resulting in the formation of an intramolecular disulfide bond (Fig. 1). Although the resolving cysteines of typical and atypical 2-Cys Prxs are not conserved in sequence, they are functionally equivalent. To recycle the disulfide, known atypical 2-Cys Prxs appear to use thioredoxin as an electron donor [26].

The last class of Prxs, the 1-Cys Prxs, conserve only the peroxidatic cysteine and do not contain a resolving cysteine (Fig. 1) [17]. Their cysteine sulfenic acid generated on reaction with peroxides is presumably reduced by a thiol-containing electron donor, but the identity of this redox partner is not yet clear (although proposed electron donors have included glutathione, lipoic acid and cyclophilin [3,7,28,29]). By analogy, one donor thiol probably forms a transient mixed disulfide bond with the enzyme, followed by its reduction by a second donor thiol, thus recycling the enzyme.

Recently, studies of several typical 2-Cys Prxs have revealed dramatic changes in oligomeric state (dimers and decamers) linked to changes in redox state like those occurring during the catalytic cycle. A combination of biophysical techniques has been used to examine the various oligomeric forms of several these enzymes, revealing an intimate connection between the oxidation state of the peroxidatic cysteine and the preferred oligomeric state of the enzyme. Although we are at an early stage of understanding the link between the oligomeric state of Prxs and their function, the recent biophysical studies have provided some important insights to the field and are the subject of this article.

Dimers, decamers and redox-dependent oligomerization

The first reports of Prx oligomerization came in the late 1960s, when transmission electron microscopy (TEM) studies of torin, an abundant protein isolated from human erythrocytes, revealed discrete complexes with apparent tenfold symmetry (Fig. 2a) [30]. Later, in the 1980s, bacterial and yeast Prxs were identified based on their antioxidant properties [31,32]. Torin has since been identified as mammalian PrxII, a typical 2-Cys Prx [33]. In the TEM reports, it was observed that under certain conditions, PrxII and the related PrxIII could also form higher-order multimers by stacking into columns of various lengths (Fig. 2b) [33,34]. The physiological relevance of these columns, if any, is not known. Recently, single-particle TEM analysis of negatively stained PrxII particles enabled the three-dimensional reconstruction of the toroid to ~20-Å resolution (Fig. 2c) [33]. Importantly, the surface-rendered TEM reconstruction correlated well with the solvent-accessible surface revealed by the X-ray crystal structure of PrxII (Fig. 2d) [20].

The oligomeric properties of several typical 2-Cys Prxs in solution have been studied using gel filtration [35–39], light scattering [22,37] and analytical ultracentrifugation [20,22]. Factors shown to promote oligomerization in typical 2-Cys Prxs include high [37] or low [34,39] ionic strength, low pH [40], high magnesium [34] or calcium [41,42] concentrations, reduction of the redox-active disulfide center [20,22,36], and 'overoxidation' of the peroxidatic cysteine to a sulfinic acid ($Cys–SO_2H$) [20]. Reduction of the active-site disulfide of typical 2-Cys Prxs is emerging as the primary factor in the stabilization of the decameric forms of these enzymes; a direct link between redox state and oligomerization state was recently established through analytical ultracentrifugation of several bacterial 2-Cys Prxs [22,43] (L.B. Poole, unpublished) and human PrxII [20], as well as earlier gel-filtration

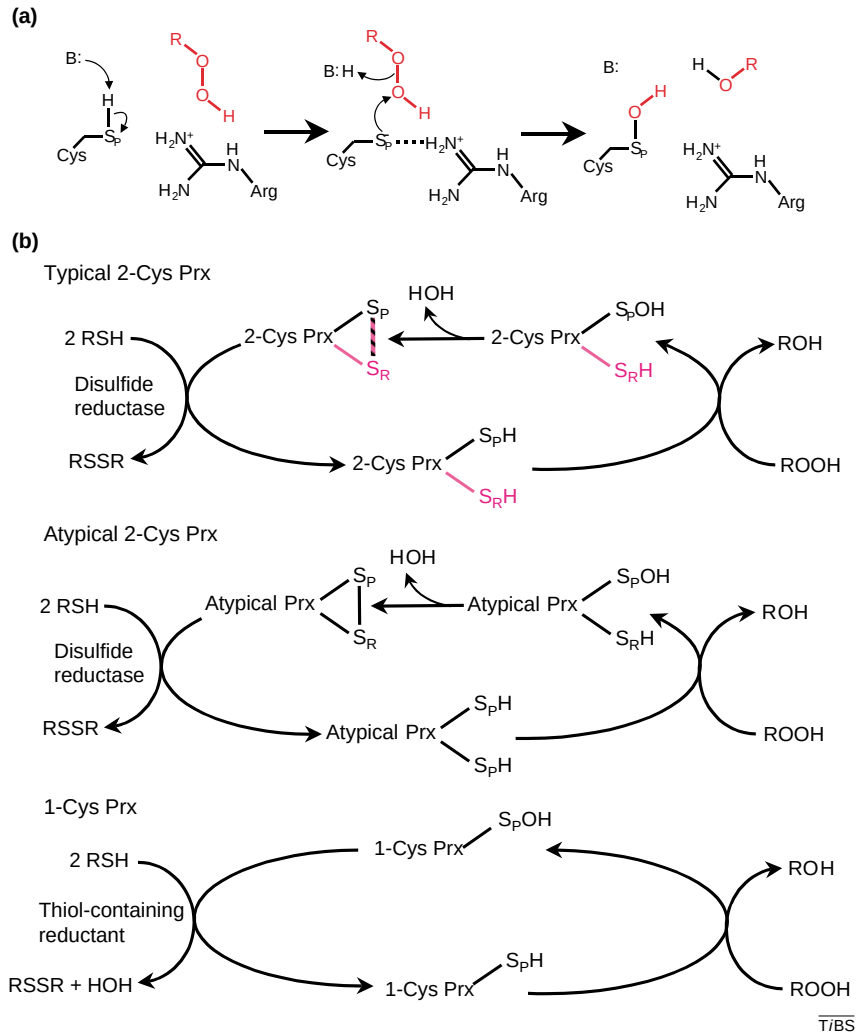

Fig. 1. Peroxiredoxin (Prx) mechanism. (a) The common first step of peroxide reduction involving nucleophilic attack by the peroxidatic cysteine (S_P) and formation of the cysteine sulfenic acid intermediate (S_POH), probably shared by all Prxs. Both the catalytic base that deprotonates the peroxidatic cysteine and the catalytic acid that protonates the RO^- leaving group are labelled 'B', although this does not imply that they are the necessarily same entity. The guanidino group of the conserved arginine is presumed to stabilize the ionized peroxidatic cysteine. (b) The three mechanisms distinguishing the Prx classes, with peroxidatic cysteines and resolving cysteines in the reduced (S_PH and S_RH, respectively), sulfenic-acid (S_POH) or disulfide (S_P and S_R connected) state. In the case of dimeric 2-Cys Prxs, the peroxidatic cysteine (black S_P) and resolving cysteine (pink S_R) originate from different subunits and condense to form an intersubunit disulfide bond (black and pink striped bar). Reduction of typical and atypical 2-Cys Prxs involves one flavoprotein disulfide reductase and at least one additional protein or domain containing a CXXC motif, which is oxidized from a dithiol (2 RSH) to a disulfide (RSSR) state during Prx reduction (e.g. thioredoxin reductase and thioredoxin, AhpF, trypanothione reductase, trypanothione and tryparedoxin, or lipoamide dehydrogenase, SucB and AhpD [23–25]). Reductants of 1-Cys Prxs include low molecular weight thiols, but physiological partners are as-yet unidentified.

studies of porcine PrxII [36]. Information gleaned from high-resolution crystal structures of various redox and oligomeric forms of Prxs is shedding light on the mechanisms controlling the redox-sensitive oligomerization.

The Prx classes have similar active sites

Since 1998, the crystal structures of six Prxs have been published, including four typical 2-Cys Prxs (PrxI, PrxII, TryP and AhpC [19–22]), one atypical 2-Cys Prx (PrxV [27]) and one 1-Cys Prx (PrxVI [17]) (Fig. 3). These structures reveal Prxs to be very similar, each containing a thioredoxin fold with a few additional secondary-structure elements present as insertions. The most striking differences involve their oligomeric states. The atypical 2-Cys Prxs are monomeric enzymes, whereas both the typical 2-Cys and the 1-Cys Prxs are domain-swapped homodimers in which the C terminus of one subunit reaches across the dimer interface to interact with the other subunit. In the typical 2-Cys Prxs, the resolving cysteine is located in this C-terminal arm. Interestingly, three of the typical 2-Cys Prxs crystallized as toroid-shaped complexes consisting of a pentameric arrangement of dimers [an $(\alpha_2)_5$ decamer], consistent with observations that 2-Cys Prx dimers can form discrete higher-order oligomers. AhpC from *Amphibacillus xylanus*, another 2-Cys Prx, also crystallizes as an $(\alpha_2)_5$ decamer [37], although no crystal structure for this enzyme has been reported.

The structure and sequence of the peroxidatic active site is highly conserved among the Prx classes. In the reduced (SH) form, the peroxidatic cysteine is in a narrow, solvent-accessible pocket formed by a loop-helix structural motif (Fig. 4a). The cysteine is located in the first turn of the helix and is surrounded by three residues conserved in all classes – Pro44, Thr48 and Arg127 (PrxII numbering).

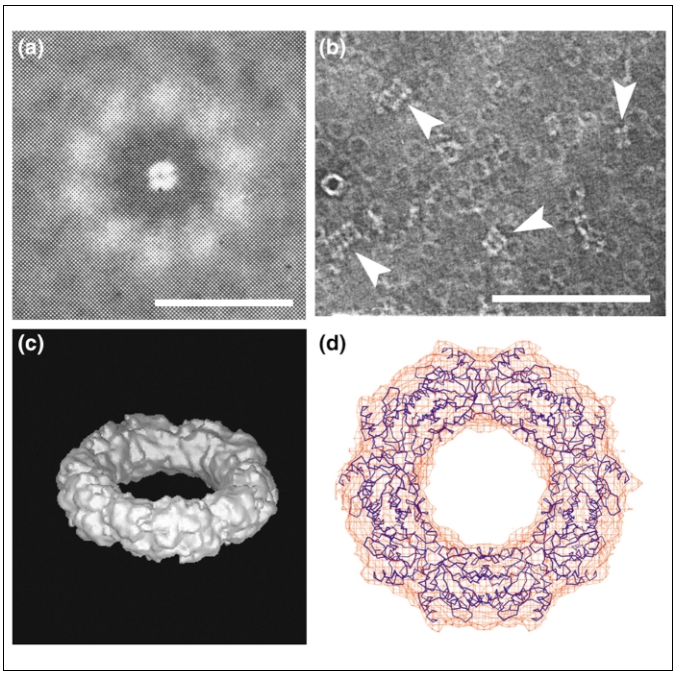

Fig. 2. Transmission electron microscopy studies of Peroxiredoxin (Prx II). (a) A two-dimensional projection of a single torus of erythrocyte Prx II (torin). Contrast was enhanced by a tenfold rotation and superposition of the images. Scale bar, 10 nm. Reproduced with kind permission from *J. Mol. Biol.* [30]. (b) PrxIII from bovine mitochondria [34,35], prepared as described in Ref. [54]. Single and stacked decamers (arrowheads) are shown. Scale bar, 100 nm. (c) Surface-rendered three-dimensional reconstruction of PrxII at 20 Å resolution. Reprinted with permission from *Biochim. Biophys. Acta* [33]. (d) The Cα trace of the 1.7-Å resolution crystal structure of erythrocyte PrxII fitted to the electron density of the 20-Å transmission electron microscopy three-dimensional reconstruction [33]. This figure was generated using MOLSCRIPT [55].

The pyrrolidine ring of Pro44 limits the solvent and peroxide accessibility of the peroxidatic cysteine and shields the reactive cysteine sulfenic acid intermediate from further oxidation by peroxides. Although the side chain of residue 45 is not conserved, its main-chain conformation is, resulting in its peptide amide donating a hydrogen bond to the Sγ of the peroxidatic cysteine (Cys51), whereas its carbonyl oxygen accepts a hydrogen bond from the Oγ of Thr48 in the reduced structures (PrxV and TryP) (Fig. 4a).

The Thr48 Oγ also accepts a hydrogen bond from the Sγ of the peroxidatic cysteine. The short hydrogen-bonding distance of 3.0 Å is good evidence that the peroxidatic cysteine is protonated, at least in the current PrxV crystal structure; otherwise the proximity of the negatively charged thiolate and the lone pair of electrons on Thr48 Oγ represents a very unfavorable electrostatic interaction. Still, the pK_a of the peroxidatic cysteine is presumably lower than that of a free cysteine (pK_a ~ 8.5) because of the nearby guanidino group of the conserved Arg127. The Thr might position the thiol proton for abstraction by an unidentified catalytic base, and the Arg might aid this by stabilizing the growing negative charge on the sulfur. The protonated peroxidatic cysteine might also be favored by the relatively low pH (~5.3) of the PrxV crystals; at higher pHs, the thiolate would dominate and the position of the Thr would be adjusted to accommodate the nearby negative charge.

Both Thr48 [44] and Arg127 [45] have been shown to be required for catalysis through mutational studies, although the Thr → Ser mutant does retain some activity. Because the peroxidatic and resolving cysteines in both typical and atypical 2-Cys reduced Prxs are more than 10 Å apart and are oriented in opposite directions, substantial structural rearrangements of both the loop–helix motif and the C-terminal arm of 2-Cys Prxs are necessary to form a disulfide bond. The active-site conformation observed for reduced Prxs is also seen in the structures in which the peroxidatic cysteine is oxidized to sulfenic [–SOH (PrxVI)] and sulfinic [–SO_2H (PrxII)] acids.

The 1-Cys Prx (PrxVI) crystal structure [17] reveals the peroxidatic cysteine as a sulfenic acid (–SOH), which is stable in 1-Cys Prxs but an intermediate in the path to disulfide-bond formation in 2-Cys Prxs. Although the loop-helix structure (Fig. 4a) is still present, the sulfur atom is shifted, disrupting the hydrogen bonds to the arginine and the peptide amide, and forming a new hydrogen bond to a nearby histidine [17]. This histidine is not conserved in all Prxs, suggesting that if this is an important interaction, it is only so for this particular class of enzyme. The sulfur atom is buried by the sulfenate oxygen and is no longer solvent accessible, presumably preventing its overoxidation to inactive sulfinic (–SO_2H) and sulfonic (–SO_3H) acids. Still, overoxidation of some Prxs has been reported.

This burial of the peroxidatic cysteine sulfenic acid raises the question of how a thiol-containing reductant can access the sulfur atom to recycle the enzyme. It is possible that a local unfolding of the active site exposes the cysteine to accomplish this. Such an unfolding is seen in the disulfide-bonded form of 2-Cys Prxs (PrxI and AhpC), in which the helical portion of the loop–helix motif is unwound, exposing the peroxidatic cysteine (Fig. 4b). The C-terminal arm is also unfolded in these structures, positioning the resolving cysteine for disulfide-bond formation. The resulting disulfide bond is solvent exposed at one end, allowing the resolving cysteine to be attacked by thiol-containing reductants [46]. In crystal structures of the disulfide-bonded enzymes (PrxI and AhpC), the C-terminal arm is disordered beyond the resolving cysteine, indicating a high degree of mobility for this locally unfolded segment.

Catalytic cycle for typical 2-Cys Prxs

Some typical 2-Cys Prxs from bacteria [15,34] (L.B. Poole, unpublished) and human and rat PrxII [13,27] undergo redox-sensitive oligomerization. These studies revealed that the reduced or overoxidized forms of the enzyme favored the decameric state, whereas the disulfide-bonded forms existed predominantly as dimers. The current ensemble of Prx structures forms the basis of a detailed catalytic cycle that includes the redox-sensitive oligomerization of these 2-Cys Prxs [22], although the precise nature of the link between oligomerization state and catalytic turnover is still uncertain. Two conserved sequence motifs have been identified that are necessary for decamer formation in 2-Cys Prxs: region I and region II (Fig. 4a). Region I is part of the conserved loop–helix active-site motif and plays a key role in decamer formation by forming a surface complementary to region II of

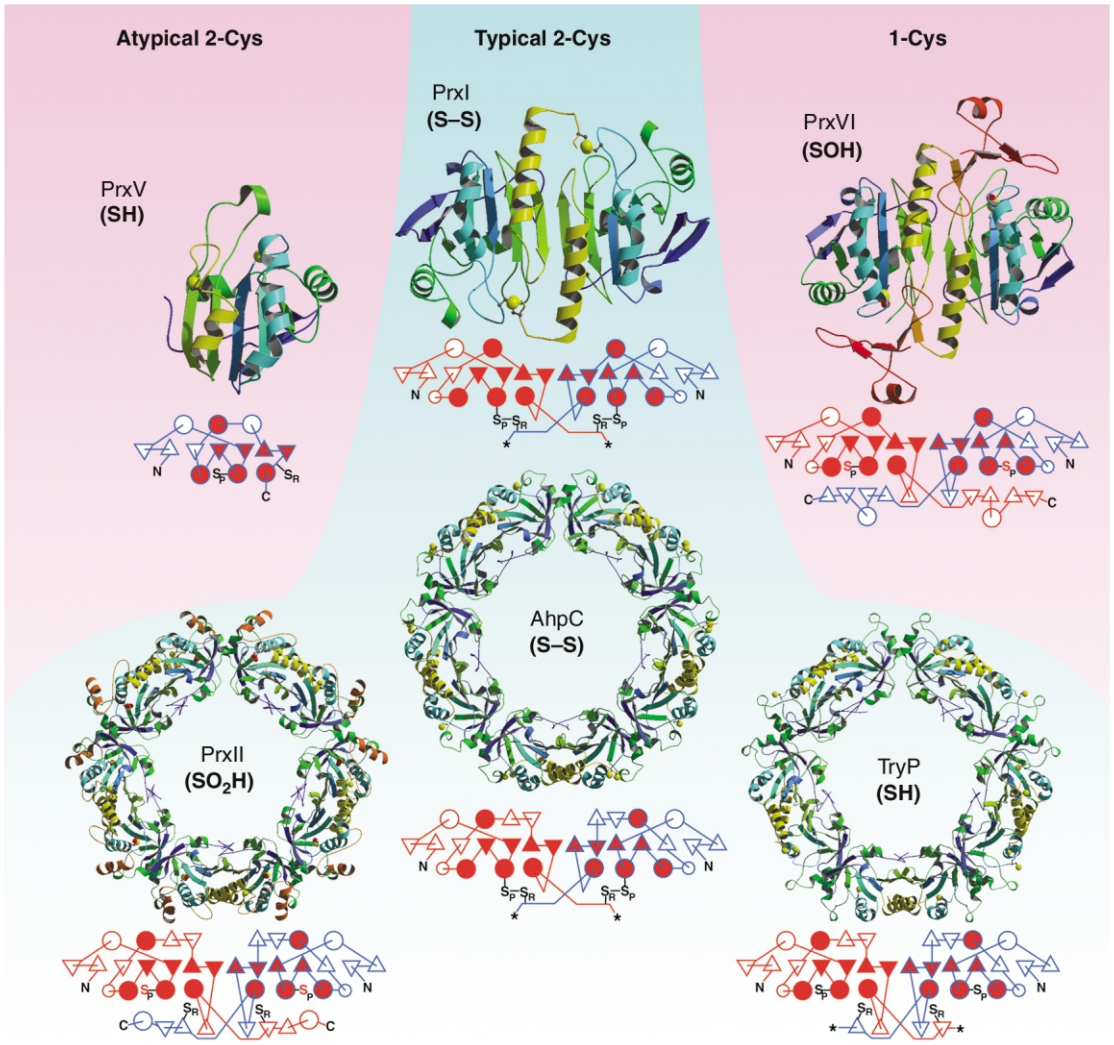

Fig. 3. Peroxiredoxin (Prx) structural classes. Ribbon models and associated topology diagrams for the crystal structures representing the three Prx classes: the typical 2-Cys Prxs (rat PrxI [19], human PrxII [20], *Crithidia fasciculata* TryP [21] and *Salmonella typhimurium* AhpC [22]); the atypical 2-Cys Prxs (human PrxV) [27]; and the 1-Cys Prxs (human PrxVI) [17]. Ribbon models are progressively colored from N to C terminus (blue to red) with consistent coloring for conserved structural elements. The topology diagrams (N and C for N and C terminus, respectively) depict the basic functional unit as domain-swapped dimers (red and blue lines for subunits) or a monomer in the case of PrxV. The conserved thioredoxin fold is identified as red-filled circles and triangles for helices and strands, respectively. The peroxidatic cysteine is identified as thiol (black S_P), sulfenic- or sulfinic-acid states (red S_P), or as a disulfide (connecting bar) with the resolving cysteine (S_R). The oxidation state of the peroxidatic cysteines of the different structures is identified in parentheses as thiol (–SH), sulfenic acid (–SOH), sulfinic acid ($-SO_2H$) or disulfide (–S–S–). Asterisks signify the truncation of the model owing to disorder. This figure was generated using MOLSCRIPT [55] and TOPS [56].

the adjacent dimer (Fig. 5a). The remaining portion of the loop–helix motif, the Cp loop (Fig. 4a), contains the peroxidatic cysteine and has been identified as the molecular switch responsible for the redox-sensitive oligomerization of 2-Cys Prxs [22].

In the reduced (thiol) state, the Cp loop forms a helix, positioning the peroxidatic cysteine in the active-site pocket and packed against region I, thus buttressing region I against region II in the interface (Fig. 5bi). During peroxide decomposition, the peroxidatic cysteine is oxidized to a cysteine sulfenic acid, burying the sulfur atom with the sulfenic-acid oxygen (Fig. 5bii). Local unfolding of the active site converts the Cp loop into a solvent-exposed loop, making the sulfenate sulfur accessible for disulfide-bond formation (Fig. 5biii). It is notable that such an unfolding in the absence of a suitable reductant could have deleterious consequences by making the sulfenate accessible to further oxidation by peroxide to yield inactive sulfinic ($-SO_2H$) or sulfonic ($-SO_3H$) acid forms (Fig. 5bvi). By maintaining a locally high concentration of reduced thiol (the resolving cysteine), 2-Cys Prxs can avoid this fate by forming a stable disulfide bond (Fig. 5biv).

Still, overoxidation of some yeast Prxs and mammalian Prxs I–IV and VI has been observed after exposure of cells to peroxides [35,47,48]. In the crystal structure of PrxII, the peroxidatic cysteine is present as cysteine sulfinic acid ($Cys-SO_2H$), trapping this protein as a decamer owing to its inability to form a disulfide bond. Overoxidation of this protein might even promote further aggregation (Fig. 2b) [34]. In this structure, the Cp loop containing the peroxidatic cysteine sulfinic acid maintains a fold very similar to that of a reduced Prx and also buttresses the dimer–dimer interface. In addition, the folded conformation of the Cp loop in the PrxII structure might

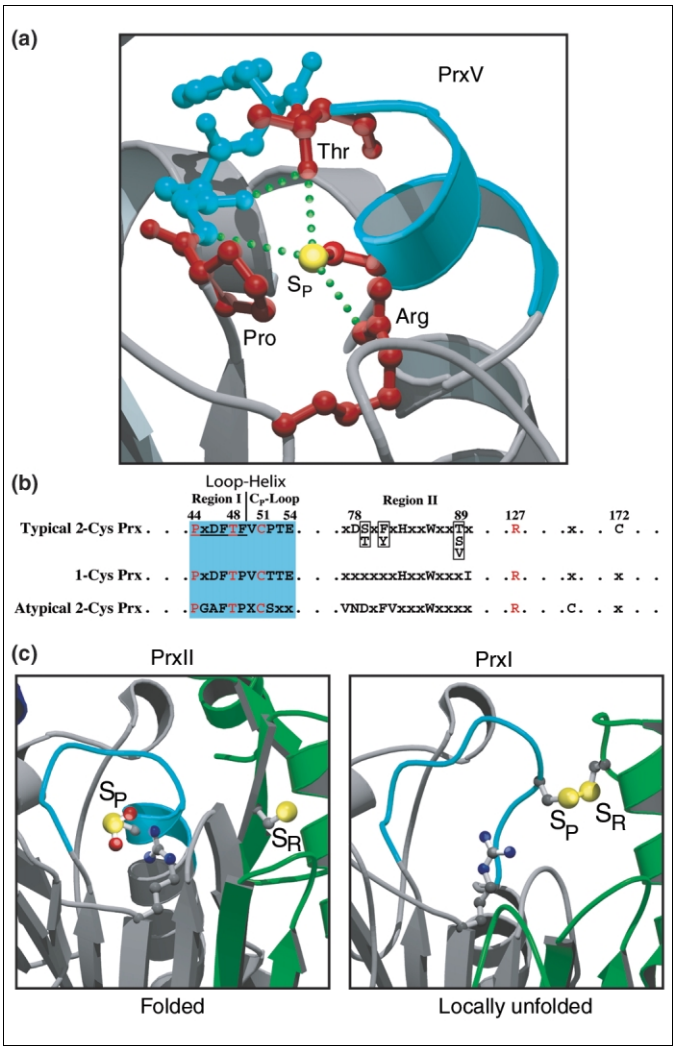

Fig. 4. Peroxiredoxin (Prx) active sites. (a) The reduced Prx active site (PrxV) and the conserved hydrogen-bonding network (dotted lines). (b) The illustration is an alignment of selected residues representing the consensus sequences for the three Prx classes. In both the illustration and the alignment, the active-site residues conserved in all Prxs are colored red and the loop-helix region blue. Also identified in the alignment are regions I and II, which are necessary for decamer formation. (c) The dramatic conformational changes in which the conserved loop–helix motif (cyan) undergoes a local unfolding required for disulfide-bond formation. The figure shows PrxI and PrxII, dimeric 2-Cys Prxs (individual monomers colored gray or green) in which the peroxidatic cysteine (S_P) is present as an overoxidized sulfinic acid (ball and stick) or in a disulfide with the resolving cysteine (S_R). The conserved Arg is also shown to ease comparisons. It is notable that the sulfinic acid form still adopts the folded conformation seen in the thiol form of Prxs.

be favored by the salt bridge between the sulfinic acid and the conserved Arg127 in the active site [20]. Interestingly, PrxII shows a well-ordered helix at its C terminus, which has been proposed to stabilize the C-terminal arm, reducing its mobility [20]. Because disulfide-bond formation requires the C-terminal arm to unfold, it was proposed that this added stability might slow down this step and allow further oxidation of the reactive Cys–SOH species [22]. The predicted influence of C-terminal-tail interactions on susceptibility to overoxidation is supported by experimental observations of a typical 2-Cys Prx from *Schizosaccharomyces pombe*, in which C-terminal truncation converts the overoxidation-sensitive enzyme to an overoxidation-resistant form [49].

In the above model, the Cp loop acts a lynchpin holding the decamer together. In the folded conformation, it stabilizes the decamer interface, whereas disulfide-bond formation traps the Cp loop in the unfolded state, in effect removing the lynchpin supporting the interface and weakening the decamer (Fig. 5biv). Despite the instability of the disulfide-bonded form of the decamer, the high protein concentration during the crystallization of this form of AhpC favored decamer formation, allowing this important intermediate to be trapped [22]. At physiologically relevant concentrations, as more active sites in the decamer form disulfide bonds, the instability reaches a critical point and the decamer breaks down into free dimers (Fig. 5bv). In doing so, region I collapses into the active-site pocket vacated by the peroxidatic cysteine, restructuring the oligomerization interface. The catalytic cycle is completed with the reduction of the redox-active disulfide bond. Although the loop-helix structure of the active site is conserved in all Prxs, the region-I–Cp-loop and region-II sequence motifs have only been identified in typical 2-Cys Prxs (Fig. 4b). Given this sequence conservation and the observation that typical 2-Cys Prxs from groups as diverse as bacteria and mammals have been reported to undergo redox-sensitive oligomerization, it is tempting to speculate that this might be a property of this class in general.

Regulation of Prx activity

Prxs have received a great deal of attention recently owing to their role in regulating levels of hydrogen peroxide, an intracellular signaling molecule common to many cytokine-induced signal-transduction pathways [3,8,12,13]. As noted above, some Prxs are themselves sensitive to inactivation by hydrogen peroxide and perhaps peroxynitrite through irreversible oxidation of their peroxidatic cysteine. Indeed, regulation of redox signaling through cysteine modification by peroxides and peroxynitrite has been reported for a growing number of enzymes and transcriptional regulators [50]. It was recently shown that the overoxidation of PrxII is likely to be physiologically relevant, in that its peroxidatic cysteine is oxidized to sulfinic ($-SO_2H$) or sulfonic ($-SO_3H$) acid forms *in vivo* upon exposure of Leydig cells to tumor necrosis factor [51]. It has been proposed that Prxs in mammalian cells act as a dam against oxidative stress, and that the ratio of active to inactive enzyme might play a role in whether cells are susceptible to cytokine-induced apoptosis [51]. In addition to overoxidation, Prx activity has also been shown to be regulated by phosphorylation and proteolysis [36,49,52,53].

Recently, phosphorylation of mammalian PrxI, PrxII, PrxIII and PrxIV at the conserved residue Thr89 (PrxII numbering) by cyclin-dependent kinases was shown to decrease the peroxidase activity of the Prxs [52]. In the case of PrxI, this phosphorylation was observed to occur *in vivo* during mitosis. The authors concluded that the phosphorylated Thr89 had an unfavorable electrostatic effect on the peroxidatic active site. However, analysis of the mammalian crystal structures show Thr89 to be solvent exposed and too distant (>16 Å) to interfere with the active site. An examination of the structure of the PrxII decamer reveals that a phosphorylated Thr89 would introduce unfavorable electrostatic interactions within the dimer–dimer interface by placing two negatively

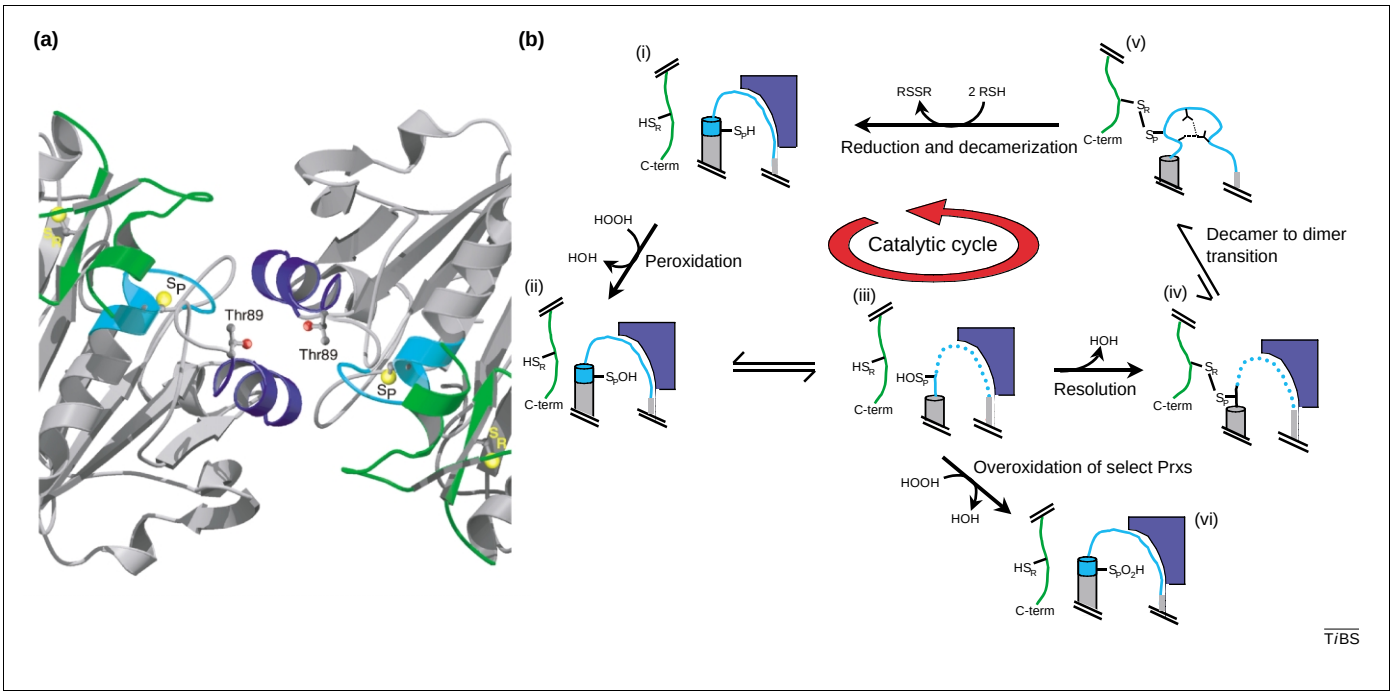

Fig. 5. Redox-sensitive oligomerization. (a) The interface between adjacent dimers in the Peroxiredoxin (Prx) II decamer. The loop-helix (including region I), region II and the C-terminal arm (C-term) are colored cyan, blue and green, respectively. Also depicted in ball and stick are the peroxidatic cysteine (S_P), the resolving cysteine (S_R), and Thr89, the site of phosphorylation by regulatory kinases [52]. (b) The catalytic cycle of some typical 2-Cys Prxs with relevant species indicated by roman numerals. The loop-helix, region II and the C-terminal arm are colored as in (a). The different oxidation states of the peroxidatic cysteine are identified as thiol [S_PH; species (i)], sulfenic [S_POH; species (ii) and species (iii)] or sulfinic [S_PO_2H; species (vi)] acid forms, or disulfide bonded with the resolving cysteine [S_R; species (iv) and (v)]. The loop-helix (cyan) is depicted in folded [cylinder and solid line; species (i), (ii) and (vi)] or unfolded [dashed line; species (iii) and (iv)] conformations, or with the peroxidatic active site restructured [species (v)]. Unidirectional arrows indicate changes in redox state and bidirectional arrows represent dynamic equilibrium.

charged phosphates in close proximity (Fig. 5a). Indeed, a reasonable alternative interpretation is that phosphorylation of Thr89 attenuates the enzyme activity by disrupting the decameric structure (Fig. 5a). Several researchers have reported that dimeric forms of Prxs exhibit less activity than decameric forms [23,37,39]. This observation is supported by the crystal structures, which show that the active sites of the typical 2-Cys Prxs are adjacent to and stabilized by the dimer–dimer interface of the decamer. It is notable that these two control mechanisms, phosphorylation and overoxidation, probably favor different oligomeric states (dimer and decamer, respectively).

Another mechanism proposed to regulate peroxidase activity *in vivo* entails specific proteolysis of the C termini of Prxs, preventing peroxide-mediated inactivation in response to rising levels of peroxide [49]. In studies of a typical 2-Cys Prx from yeast, a portion of the enzyme was found to have a truncated C-terminal following purification [49]. In follow-up mutagenesis studies, C-terminally truncated forms of the enzyme were found to be more resistant to peroxide overoxidation and inactivation than the sensitive wild-type enzyme [49]. A similar truncation of PrxII that removed the C-terminal 13 residues (including the last α helix) has also been observed during the isolation of the enzyme from erythrocytes [53]. Interestingly, the regulatory protease calpain is present in erythrocytes and will specifically cleave this region of PrxII *in vitro* [36]. Proteolysis would make the enzyme resistant to overoxidation but leave it susceptible to inactivation by phosphorylation.

Conclusions

The ubiquitous Prxs appear to be diverse in function, ranging from antioxidant enzymes to regulators of signal transduction. This diversity is reflected in slight evolutionary modifications in sequence and structure, built around a common peroxidatic active site. The literature within the Prx field is currently focused on their more recently identified roles as regulators of redox-sensitive signaling [3,8]. Although the precise relationship between the peroxidase activity and the oligomeric status of these enzymes is currently unclear, the two appear to be closely linked. Here, we have highlighted the current state of our understanding of Prx mechanism, structure and regulation.

- There are three classes of Prx, distinguished by the number and location of catalytic cysteines – the typical 2-Cys, atypical 2-Cys and 1-Cys Prxs.
- Despite differences in quaternary structure and catalytic cycle, all three classes share the same peroxidatic active-site structure.
- Some bacterial and mammalian typical 2-Cys Prxs undergo redox-sensitive oligomerization, and this might be a property of typical 2-Cys Prxs in general.
- Prx peroxidase activity might be regulated *in vivo* by cysteine oxidation, phosphorylation and limited proteolysis.

Future research should aim to improve our understanding of the influence of changes in oligomeric structure and post-translational modifications upon the peroxidatic and signaling activities of Prxs.

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