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Overview of Peroxiredoxins in oxidant defense and redox regulation

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Abstract

Peroxiredoxins are important hydroperoxide detoxification enzymes, yet have only come to the fore in recent years relative to the other major players in peroxide detoxification, heme-containing catalases and peroxidases, and glutathione peroxidases. These cysteine-dependent peroxidases exhibit high reactivity with hydrogen peroxide, organic hydroperoxides and peroxynitrite and play major roles not only in peroxide defense, but also in regulating peroxide-mediated cell signaling. This overview focuses on important peroxiredoxin features that have emerged over the past several decades with an emphasis on catalytic mechanism, regulation and biological function.

Keywords

peroxidases; antioxidants; antioxidant enzymes; sulfenic acids; hydroperoxides; hydrogen peroxide; thiol peroxidase; Prx; PRDX; redox regulation

Introduction

Peroxiredoxins (Prxs) are a fascinating group of thiol-dependent peroxidases (EC 1.11.1.15) which detoxify H₂O₂, aliphatic and aromatic hydroperoxides, and peroxynitrite (Dubuisson, et al., 2004, Flohé, et al., 2010, Poole, 2007). They are ubiquitously expressed, with multiple isoforms present in most organisms (e.g., 3 isoforms in *Escherichia coli*, 5 in *Saccharomyces cerevisiae*, 6 in *Homo sapiens* and 9 in *Arabidopsis thaliana*) (Dietz, 2011, Knoops, et al., 2007). Many exhibit very fast rates of peroxide reduction on the order of 10⁶ to 10⁸ M⁻¹ s⁻¹ using a conserved active site architecture that is highly specialized for peroxide reduction, as their reactivity with other thiol reagents is only modest (Cox, et al., 2009, Dubuisson, et al., 2004, Horta, et al., 2010, Manta, et al., 2009, Nelson, et al., 2008, Parsonage, et al., 2010, Parsonage, et al., 2005, Peskin, et al., 2007, Stacey, et al., 2009, Trujillo, et al., 2007). While the pK_a of the active site Cys is strongly influenced by electrostatic environment and is lowered in Prxs to promote thiolate formation (values around or below ~6), this feature is quite insufficient to explain the rapid catalytic rates (Flohé, et al., 2010, Manta, et al., 2009, Nelson, et al., 2008, Winterbourn, 2008). The high catalytic efficiency as well as general high abundance of Prx protein in cells makes them the predominant scavengers of peroxides in many circumstances (Adimora, et al., 2010, Winterbourn, 2008), and it is increasingly recognized that they play major roles in the

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INTERNET RESOURCES

<http://www.csb.wfu.edu/prex/>: PREX is a searchable database containing > 6,000 Prx protein sequences unambiguously classified into one of six distinct subclasses. Subfamily classifications use information around the active sites of structurally characterized subfamily members to search for sequences with conserved functionally-relevant motifs (Nelson, et al., 2011, Soito, et al., 2011).

detoxification of and defense against potentially damaging oxidants, the same oxidants that regulate and mediate cell signaling processes (Flohé, 2010, Fomenko, et al., 2008, Hall, et al., 2009, Poole and Nelson, 2008).

The structural context and biophysical properties that underlie Prx function are also fascinating aspects of this group of proteins. Prxs are built on a thioredoxin (Trx) scaffold, like glutathione peroxidases (Gpxs), and rely on an active site cysteine within a PxxxTxxC motif for catalysis. Structural and bioinformatic evidence supports the idea that the canonical CxxC redox motif in Trxs and glutaredoxins (Grxs) (with the first Cys acting as the nucleophile in thiol-disulfide interchange) has diverged to become both the TxxC motif of Prxs and the CxxT (or UxxT, with U = selenocysteine) of Gpxs (Fomenko and Gladyshev, 2003). This change results in the gain of peroxidase activity and the loss of the more general protein disulfide reductase functions. Intriguingly, one additional residue required for catalysis which lies outside the active site loop/helix region, an Arg residue contributing to the Prx active site from about 75 residues away in sequence, is in a position equivalent to that of the conserved *cis*-Pro in the reductases (Copley, et al., 2004, Su, et al., 2007).

Bioinformatics tools applied to gain insight into the key amino acids and interactions underlying catalytic function have also provided information about the subfamilies into which members of this broad and diverse Prx family can be subdivided (Copley, et al., 2004, Nelson, et al., 2011). Structural information is also increasingly available and has provided additional opportunities to investigate the important features of Prxs (Hall, et al., 2011).

This unit provides an overview of the important Prx features that have emerged over the past several decades with an emphasis on catalytic mechanism, regulation and biological function.

The common and distinct features of peroxide reduction and enzyme recycling among the Prx subfamilies

The Catalytic Cycle

Accumulating information in the early 90's pointed to the essentiality of a single Cys residue for catalysis of peroxide reduction by Prxs. It was noted that a second Cys was often, but not always, present and conserved near the C-terminus, suggesting a mechanistic distinction between these "2-Cys" and "1-Cys" groups of Prx enzymes (Chae, et al., 1994). We now recognize the absolutely conserved Cys residue as the "peroxidatic" Cys (denoted C_p or S_p for the sulfur atom) that attacks the hydroperoxyl substrate, forming the first product (water or alcohol in the case of H₂O₂ or larger ROOH substrates, respectively) and a sulfenic acid moiety on the active site C_p residue (Fig. 1). This chemistry matches the now well established mechanism of H₂O₂ reduction by the single-Cys containing NADH peroxidase flavoenzymes from gram positive lactic acid bacteria (Crane, et al., 1997, Poole and Claiborne, 1989, Yeh, et al., 1996).

In a minimal catalytic mechanism for Prxs, sulfenic acid is "captured" (or resolved) by a thiol group, generating H₂O as the second product and a disulfide bond in the enzyme on the pathway to reductive recycling (Fig. 1). If the thiol group comes from the Prx, this Cys residue is called the "resolving" Cys or C_r; in the earliest studies of Prxs (Chae, et al., 1994, Chae, et al., 1994), this residue within the C-terminus was noted to come from another subunit of a dimer, generating an intersubunit disulfide bond with the C_p (two active sites and two disulfides per α₂ dimer). In the subsequent years it has been increasingly recognized that the C_r can be contributed from a number of other positions within the same subunit, as well (sometimes designated the "atypical" 2-Cys Prxs) (Wood, et al., 2003). In the 1-Cys

Prxs, the resolving thiol must come from a non-Prx molecule which may be another protein or even a small molecule reductant like glutathione. While this 1-Cys mechanism was first described for human PrxVI¹, the first Prx to be structurally characterized (Choi, et al., 1998), it is now recognized to apply to Prxs within other subfamilies that lack a C_r, as well.

The most common reductant used for recycling by members of all subfamilies of Prxs is Trx (where both R" SH moieties of Fig. 1 come from a single Trx CxxC active site). Other reductases similar to Trx (e.g. trypanothione from kinetoplastids, the Grx-like Cp9 of *Clostridium pasteurianum*, the N-terminal domain of bacterial AhpF, and plant NTR) are also found to act as specialized Prx reductases in certain organisms (Dietz, 2011, Flohé, et al., 2010, Poole, et al., 2000). The nature of recycling of human PrxVI and many other 1-Cys Prx enzymes *in vivo* continues to be a matter of some debate; evidence has been reported for the involvement of glutathione and glutathione transferase pi (GSTπ), lipoic acid, ascorbate, cyclophilins, and reductases including Grxs and Trxs (Dietz, 2011, Flohé, et al., 2010).

Localized unfolding and refolding for catalysis

Resolution of the Prx sulfenic acid, regardless of mechanism, requires a localized unfolding of structures around the C_p (often accompanied by structural changes around the C_r, when present) that removes the S_pOH from the "fully folded" (FF) active site and exposes it (in the "locally unfolded" or LU form) for disulfide bond formation with C_r or another resolving thiol group. This essential step occurs with varying efficiencies and can lead to the persistence of the SOH within the FF active site of some Prxs, providing an opportunity for oxidative regulation during the catalytic cycle (Fig. 1), an issue which is discussed in more detail below.

Prx subfamilies

Based on the variation in the location and even presence of C_r in different Prxs as well as notable structural distinctions between them, it is clear that different classes of proteins have arisen during Prx evolution, all of which maintain the common peroxide reduction chemistry and active site architecture, but vary in the details of their recycling pathways (Hall, et al., 2011). Moreover, features such as oligomeric state and propensity toward oxidative inactivation during turnover also vary among different Prxs. To better explore the distinctions between such subclasses and take advantage of the large amounts of genomic sequence information currently available, various bioinformatics approaches have been applied (Copley, et al., 2004, Dietz, 2011, Knoops, et al., 2007, Koua, et al., 2009). One recent study (Nelson, et al., 2011) utilized an approach known as functional site (or active site) profiling to identify >3500 Prx sequences from the Jan 2008 release of GenBank, each classified unambiguously into one of six Prx subfamilies that had been previously identified by other bioinformatic and structural analyses (Table 1). The strength of this approach is that classification is based not on full sequence alignments but on the conservation of conserved sequences proximal to the active site. The generation of large lists of Prx sequences with known subfamily membership, now available in a publically accessible database (<http://csb.wfu.edu/prex/>) (Soito, et al., 2011), allowed for detailed analyses of residue conservation, phylogenetic distribution and overall conservation patterns for the C_r of each subfamily, and some of the most relevant results from this work are described in the following paragraphs.

¹Herein we will use the Roman numerals as specific mammalian Prx names, as they have been used before, and Arabic for the other species and the larger subclasses, but there is no clear consensus across the field on this issue.

The “typical 2-Cys” group of Prxs comprised of the founding members of the 2-Cys Prxs described above (denoted here as the Prx1/AhpC subfamily) is a much studied and broadly distributed group of proteins that form intersubunit disulfide bonds during catalysis. This group includes as members human PrxI through IV and bacterial “AhpC” proteins (named for their alkyl hydroperoxide reductase activity). Members of both the Prx1/AhpC subfamily and the Prx6 subfamily contain a C-terminal extension relative to the ancestral Trx fold and to other Prx subfamilies (Table 1), a feature which has led to their being grouped together by some bioinformatic analyses (Copley, et al., 2004, Karplus and Hall, 2007). Despite this, active site profiling clearly distinguishes the two subfamilies. For the 2-Cys proteins in the Prx1/AhpC subfamily, the C_r is found within this C-terminal extension; although Prx6 subfamily members do in a very few cases possess a putative C_r, the majority appear to lack a C_r (Table 1, see below). Members of the Prx1/AhpC subfamily form not only α₂ dimers, brought together through interactions at the edges of their β sheets (the so-called B-type interface), but also higher order oligomeric structures [primarily (α₂)₅ decamers] built up from an alternative, A-type interface (Fig. 2) (Hall, et al., 2011). This A-type interface plays an important role in supporting catalysis in Prx1/AhpC proteins, as described in more detail below.

Two thiol-dependent peroxidases described in the mid- to late 90's, human PrxV and *E. coli* thiol peroxidase (Tpx), became founding members of two distinct Prx subfamilies (Baker and Poole, 2003, Cha, et al., 1995, Knoop, et al., 1999, Sarma, et al., 2005, Seo, et al., 2000). Members of these subfamilies are typically dimeric and form intrasubunit disulfide bonds (Table 1). Interestingly, the interface used for dimer formation in these enzymes is essentially the same A-type interface used to form higher order oligomeric structures in Prx1/AhpC proteins (Fig. 2).

Two other subfamilies have also emerged, one of which is relatively broadly distributed in biology, though lacking in animals (the BCP/PrxQ group), and the other (AhpE) which is a recent addition to the Prx family and is only narrowly represented among species related to that of the founding member, *Mycobacterium tuberculosis* (Table 1). Within the BCP/PrxQ subfamily of proteins, the low potential PrxQ proteins of plant chloroplasts have received much attention and likely have a function in photosynthesis within this organelle (Dietz, 2011). The representative from *E. coli*, BCP (which stands for bacterioferritin comigratory protein), seems to be less active as a peroxidase than most Prxs (Jeong, et al., 2000), though the functional importance of this protein has been demonstrated, at least in the human pathogen *Helicobacter pylori*, where it helps establish long term infections within the host's gastric mucosa (Wang, et al., 2005). Members of this group have generally been thought to be monomeric (Nelson, et al., 2011) though recently added structural representatives show that some members can form dimers using the A-type interface used to form dimers in the Tpx and Prx5 subfamilies (Hall, et al., 2011). The few AhpE representatives do not clearly fit into any other Prx subfamilies (Hall, et al., 2011, Nelson, et al., 2011), and only one representative from *M. tuberculosis* has been structurally and kinetically characterized as of December 2010. This protein has a significant peroxynitrite reductase activity on the order of 10⁷ M⁻¹ s⁻¹ that dominates somewhat over its peroxidase activity (Hugo, et al., 2009), and forms an A-type dimer (Li, et al., 2005). Although *M. tuberculosis* AhpE utilizes a 1-Cys mechanism, other members of this subfamily contain a potential C_r (Nelson, et al., 2011).

Location and prevalence of C_r in Prx subfamilies

For 2-Cys Prxs, the step of catalysis after peroxide reaction is resolution of the C_p-sulfenic acid by condensation with a thiol group provided by another Cys in the enzyme (C_r, or S_r in Fig. 1), forming either an intra- or intersubunit disulfide bond. As mentioned above, a remarkable feature of the Prxs is how many different sites have been shown to contain a

functionally active C_r. In two of the subfamilies, Prx1/AhpC and Tpx, the distinct location of C_r is highly conserved (within the C-terminus and helix α 3, respectively), with >96% of all members conforming to this pattern (Table 1) (Hall, et al., 2011, Nelson, et al., 2011). For Prx6 and AhpE subfamily members, the C_r may occur only rarely, with the majority of subfamily members functioning as 1-Cys Prxs. For BCP/PrxQ and Prx5 subfamilies, the presence and/or location of C_r is more variable. The C_r of human PrxV, within helix α 5, is in fact relatively uncommon in this group of proteins, with only 17% of members (mostly metazoans) possessing it; another 16% of Prx5 members, all from bacteria, are fused to a Grx domain as observed for the canonical Grx/Prx hybrid protein from *Haemophilus influenza* (Nelson, et al., 2011). Among BCP/PrxQ proteins, a slight majority (55%) have C_r located $\sim 1\frac{1}{2}$ turns down the α 2 helix from C_p; another subset of subfamily members (7%) are now recognized to use an alternate position for C_r, in helix α 3 (as is usually observed for Tpx proteins). These observations support the hypothesis that the C_r has arisen independently multiple times during the evolutionary divergence of the Prxs and also support the notion that the BCP/PrxQ group of proteins are the most representative of an ancestral form of the Prxs (Copley, et al., 2004, Hall, et al., 2011).

Chemical and structural features promoting catalysis in peroxiredoxins

While the chemistry of a thiolate group attacking the –O–O– bond of a hydroperoxide substrate seems simple at first glance, the forces that drive and regulate catalysis are many and sometimes contradictory. For example, thiolates (R-S[−]) are better nucleophiles than thiols (R-SH); the reactivity of the latter with hydroperoxides is vanishingly low. Therefore, a pK_a for the C_p of one or several pH units below the expected, unperturbed pK_a of around 8.5 would be considered supportive of catalysis through enhanced ratios of thiolate to thiol at physiological pHs. For Prxs, measured pK_a values have ranged from 5.2 to 6.3 (Table 2) (Horta, et al., 2010, Hugo, et al., 2009, Manta, et al., 2009, Nelson, et al., 2008, Ogunucu, et al., 2007). The complication of lowering the active site pK_a is, however, the fact that a low pK_a thiolate is a worse nucleophile than a high pK_a thiolate, so that stabilization of the negative-charged thiolate would seem to come at the expense of chemical reactivity. However, Prxs, which were once thought to exhibit rather poor activity, have more recently been noted for their quite high reactivities with hydroperoxide substrates, ranging upwards of 10⁷ – 10⁸ M^{−1} s^{−1}, values which are within the realm of the highly reactive selenocysteine-containing glutathione peroxidases (Cox, et al., 2009, Horta, et al., 2010, Manta, et al., 2009, Parsonage, et al., 2005, Peskin, et al., 2007, Trujillo, et al., 2007). How then can the Prx active site impart such high reactivity to this sulfur-containing residue?

Part of the answer seems to lie in the observations that the charge stabilization and hydrogen-bonding features of the active site not only promote thiolate stability but also position and thus activate the peroxide to be attacked. The active site geometry is also set up for stabilization of the transition state wherein the “O_A”, the terminal oxygen of the hydroperoxide, makes favorable interactions for weakening the bond to the “O_B” of the peroxide and for forming a new bond to the C_p sulfur atom (Fig. 3). Based on a large scale analysis of over 70 Prx structures and emerging examples or mimics of substrate and product complexes, the O_A can be envisioned as sliding along a hydrogen bond-guided “track” during catalysis, starting from a position covalently bonded to O_B 1.5 Å apart, and ending up attached to the S_p of the Cys, ~ 3 Å from O_B (Hall, et al., 2011, Hall, et al., 2010). This track is reasonably well defined structurally given the many ligand-containing Prx structures where one or two oxygen atoms occupy positions within the track that mimic O_A and/or O_B. In some cases where carboxylates and diols are bound, such as the high resolution benzoate and dithiothreitol (DTT) complexes with human PrxV (PDB identifiers 1hd2 and 3mng, respectively), two oxygens are bound along the track with atomic separations of ~ 2.4 Å, suggesting that these types of ligands act as transition-state analogs,

sampling intermediate positions along the linear oxygen track created within the Prx active site (Hall, et al., 2010).

As our view of the Prx active site has sharpened, the roles for the highly conserved residues within the active site have become clearer based on these analyses: (1) the hydroxyl group of Thr (or in rare cases Ser) positions and activates the protonated O_A atom of the incoming hydroperoxide substrate; (2) the Arg, through both charge and hydrogen-bonding interactions, positions and activates both the active site thiolate and the peroxide substrate; and (3) the Pro shields the active site from unwanted reactions and positions the backbone of the following two residues to provide additional hydrogen bonding interactions (Fig. 2) (Hall, et al., 2010).

Regulatory Oxidation and Other Post-translational Modifications

As Prxs were studied in increasing molecular detail, it became apparent that the active sites of some, particularly those from higher organisms, are surprisingly susceptible to oxidative inactivation by their own substrate (Baker and Poole, 2003, Rabilloud, et al., 2002, Yang, et al., 2002). The enzyme-associated product of this oxidation, cysteine sulfinic acid ($R-S_pO_2H$), is stabilized within the Prx active site (e.g., in human PrxII as captured by crystallography in 1qmv), although at least a few Prxs can undergo yet another hyperoxidation step to form the cysteine sulfonic acid ($R-S_pO_3H$) (Sarma, et al., 2005, Seo, et al., 2009) (Fig. 1). Using these species, a model could be generated whereby the oxygen of the sulfinic acid product from catalysis rotates so that another lone pair of electrons on the sulfur can again attack a hydroperoxide substrate within the fully folded enzyme active site; a further rotation would permit sulfonic acid formation in those enzymes for which the geometry is suitable (Sarma, et al., 2005). While both sulfinic and sulfonic acids were long believed to be irreversibly oxidized forms of sulfur within biological systems, new findings in the early 2000's demonstrated the reversibility of sulfinic acid formation in a subset of Prxs that can be repaired by the enzyme sulfiredoxin (Srx) (Biteau, et al., 2003, Lowther and Haynes, 2011). The existence of a repair system that seems to be dedicated to Prx recovery from oxidative inactivation implies an important biological role for this inactivation; the nature of how this oxidative regulatory cycle adds to the functional properties of this family of proteins is a topic of much discussion (see below). As sulfinic acids other than the C_p sulfinic acid of some Prxs have not yet been demonstrated to serve as substrates of Srx proteins, this appears to be very specialized chemistry that may only pertain to this group of enzymes.

Oxidative inactivation during turnover at submillimolar levels of H_2O_2 is observed for a number of eukaryotic Prxs, but is rare among prokaryotic Prxs (Hall, et al., 2009, Wood, et al., 2003). A structural explanation for the sensitivity lies in the architecture of the protein surrounding the active site, at least in the case of the Prx1/AhpC group of Prxs which form intersubunit disulfide bonds during the catalytic cycle. In some of the sensitive Prx1-like proteins, the FF form of the active site is stabilized by interactions between the active site surrounding the C_p and two regions in the protein with conserved sequences. Stabilization of the FF form of the active site by these surrounding interactions causes the sulfinic acid formed upon peroxide reduction to persist longer within the active site, where a second peroxide can bind and further oxidize it. The first stabilizing interaction involves a proximal GGLG-containing segment following a 3/10 helix, from the same subunit as the C_p , and the second interaction is with the C-terminal tail extending past the C_t of a second subunit, including a conserved YF motif within the helix (present in Prx1-like proteins but not bacterial AhpCs). Several lines of evidence support the role of these two regions in hyperoxidation. For example, the shorter C-terminus and absence of the inserted GGLG in bacterial AhpC support a much more flexible active site compared with human PrxII, a

sensitive Prx, and local unfolding of the active site is more favorable (Wood, et al., 2003). In this way, intersubunit disulfide bond formation is facilitated, locking in the LU conformation and removing the reactive sulfenic acid, thereby avoiding hyperoxidation. This emphasizes the important role for C_r in protection against oxidative inactivation (Ellis and Poole, 1997, Trujillo, et al., 2006). Where investigators have introduced single amino acid substitutions into sensitive Prxs (Koo, et al., 2002) or swapped C-terminal tails of sensitive and robust Prxs by mutagenesis (Sayed and Williams, 2004), predictable effects on hyperoxidation sensitivity were observed, consistent with a linkage between the propensity toward hyperoxidation during catalysis and C-terminal tail packing over the active site. Thus, while stabilization of the FF active site should promote the peroxidase reaction to a point, further stability promotes hyperoxidation, providing a “rheostat” for oxidative regulation through the C_p sulfenic acid formed during catalysis. Through evolution, this feature can therefore be “tuned” to fit the need corresponding to its biological function (Poole, et al., 2004). Interestingly, new findings suggest that further oxidation to form the sulfonic acid at C_p is also observed primarily for PrxI, but not PrxII (which becomes N-terminally acetylated in cells, unlike PrxI); this irreversible oxidation may be important as an additional regulatory step that prevents recovery through Srx-dependent repair (Seo, et al., 2009).

Other controls on catalytic efficiency and/or hyperoxidation sensitivity also involve posttranslational modifications that are probably only partially recognized at this point, though the list is steadily growing [reviewed in (Aran, et al., 2009)]. Briefly, cell cycle-dependent phosphorylation of Thr90 in PrxI was observed to occur during mitosis through action of CDK1, and this modification led to an overall decrease in activity (Chang, et al., 2002). A later paper identified a new site of phosphorylation on PrxI, Tyr194, as a modification occurring to membrane-associated PrxI, inactivating it under conditions where oxidative signaling may be occurring (see below). PrxI and II were also shown to accumulate as acetylated proteins in cells depleted of HDAC6, and this modification occurred at the penultimate residue, a Lys, within the C-terminal tail of these proteins (Parmigiani, et al., 2008). Acetylation reportedly enhances the activity of Prxs and increases their resistance to hyperoxidation (Parmigiani, et al., 2008), as does C-terminal truncation (Jara, et al., 2008, Koo, et al., 2002, Seo, et al., 2004), consistent with disruption of the packing of this region over the active site which in turn promotes reductive recycling (see next section).

Interplay between oligomerization and catalysis

As X-ray structures and biophysical characterizations of Prxs were emerging in the early 2000's, it became clear that there was an unusual link between oligomeric state, redox status and catalysis by Prxs in the AhpC/Prx1 subfamily (i.e. typical 2-Cys Prxs) (Hall, et al., 2009, Wood, et al., 2002). While one might expect a disulfide bond to be stabilizing in terms of higher order structures, this disulfide between the C_p and C_r in the oxidized Prxs (across the B-type interface) instead destabilizes the decameric structures observed in the reduced and hyperoxidized enzymes (represented by the tryparedoxin peroxidase and human PrxII structures, 1uul and 1qmv, respectively) and predominantly yields dimers (as seen in rat PrxI, 1qq2). This transition was suggested initially based on differences between some of the early crystal structures, and was subsequently confirmed through analytical ultracentrifugation studies of bacterial AhpC (Wood, et al., 2002). Disulfide bond formation not only destabilizes decamers, it also results in mobilization of the extended C-termini. The disorder in this region of the protein is responsible for a lack of electron density within crystals of proteins in LU conformation (e.g., oxidized Prx1 and AhpC proteins, 1qq2 and 1yep) for ~20 or more C-terminal residues (Hirotsu, et al., 1999, Wood, et al., 2002). Not

surprisingly, the Prx1/AhpC proteins which are hyperoxidized and cannot form the decamer-disrupting disulfide bond are also stabilized as decamers in solution.

The structural explanation for this link between active site conformation and oligomerization lies in the relative proximity of the active site to the decamer building (A-type) interface. Local unfolding of the active site, which gets “locked in” by disulfide bond formation, results in a loss in stability for the decamer, because the active site loop in the FF conformation acts to buttress the decamer-building interface. Similarly, one would expect that decamer formation and the buttressing effect of adjacent dimers would contribute to the stability of the FF form, potentially affecting catalytic activity. This hypothesis was tested through the introduction of decamer destabilizing mutations into *Salmonella typhimurium* AhpC. Disrupting mutations (e.g. T77I and T77D mutations) not only promoted dimer rather than decamer formation in both oxidized and reduced forms of AhpC, but also had the effect of decreasing the catalytic efficiency of H₂O₂ reduction by about 2 orders of magnitude, with most of the effect on K_m for peroxide (Parsonage, et al., 2005). The enzymatic activity of the primarily dimeric mutants was, however, less sensitive toward hyperoxidation during turnover, as predicted based on greater flexibility around the active site that would be caused by the absence of an adjacent dimer. Computational work has also supported the importance of decamer formation for catalytic function and the electrostatic interactions around the active site that influence C_p pK_a; decamer formation restricts the conformations available to some amino acid side chains which are expected to have direct or indirect influences on C_p reactivity in the 2-Cys Prxs of this group (Yuan, et al., 2010).

Peroxide defense and cell signaling control functions for Prxs

The biological role of *E. coli* and other bacterial AhpCs as oxidant defense enzymes is well established (Imlay, 2008) and protection of cellular macromolecules from oxidative damage is likely to be an important role for most if not all Prxs. The essentiality of Prxs in biology is highlighted by their ubiquity and abundance, as one or more representative(s) seem to be highly expressed in most organisms, with the exception of some spirochetes (i.e., those closely related to *Borrelia burgdorferi*) (Parsonage, et al., 2010, Winterbourn, 2008). However, the existence of multiple means to regulate the peroxidase activity of Prxs, particularly within higher organisms, indicates that this relatively simple “oxidant defense” view doesn’t tell the whole story. Indeed, it is becoming more and more clear in higher organisms that localized reactive oxygen species (ROS) production, and particularly H₂O₂, is intimately linked to many cell signaling pathways, and the enzymatic control of the levels of these oxidants must clearly be a key factor in their effectiveness as mediators of cell signaling processes (Flohé, 2010, Rhee, et al., 2005, Wood, et al., 2003). This highlights the inescapable overlap between the concepts of oxidative stress and redox signaling, which are often taken to represent different aspects of the redox “continuum”. Cancer cells, which often exhibit high internal oxidant levels (Szatrowski and Nathan, 1991), are also highly proliferative and in some respects resistant to the effects of oxidative damage. In fact, one route for bypassing oxidant-induced apoptosis is through the deletion of the gene for p53, a redox-sensitive transcriptional regulator that is commonly lacking in aggressive cancers (Fridman and Lowe, 2003). Cancer cells also typically exhibit an enhanced arsenal of antioxidant defense enzymes, many of which are among the repertoire under the control of Keap1/Nrf2 signaling (Kim, et al., 2008). A number of studies have linked high Prx levels with the cancerous phenotype (Lehtonen, et al., 2004, Quan, et al., 2006, Roumes, et al., 2010), with radiation resistance in cell lines (Lee, et al., 2008, Smith-Pearson, et al., 2008, Wang, et al., 2005, Zhang, et al., 2009), and with poor prognosis for chemotherapy (Iwao-Koizumi, et al., 2005, Kim, et al., 2008, Pak, et al., 2011). Nonetheless, while cancer cells may better cope with oxidants given elevated Prx expression, whole animal knockouts of PrxI, in particular, yield mice which are predisposed to development of lymphomas,

sarcomas and carcinomas (Neumann, et al., 2003). Thus, Prx proteins may act in different ways, both as tumor suppressors, since their absence leads to cancer, and as tumor promoters, where they provide important defenses against oxidative damage within the tumor environment (Neumann and Fang, 2007).

Oxidant defense provided by Prx activity does have an impact on cell signaling events, as shown most clearly in unicellular organisms. Prokaryotic, peroxide-sensing transcription factors which orchestrate the expression of defense enzymes illustrate a direct linkage between oxidant levels and signaling, while the more complex peroxide-signaling pathways in eukaryotes are likely to be regulated by Prxs in more subtle ways. Two families of bacterial transcription factors with peroxide-reactive Cys residues analogous to C_p of Prxs, OxyR and OhrR, are directly regulated by their small molecule effectors, H₂O₂ and organic hydroperoxides, respectively, using chemistry that parallels the catalytic cycle of Prxs, but with a much slower reduction step (Antelmann and Helmann, 2010, Imlay, 2008). The regulatory action of Prxs in such model organisms as *E. coli* seems well accounted for by their capacity to reduce and remove these oxidants, at least in terms of AhpC activity (Yamamoto, et al., 2008).

Nonetheless, Prxs sensitive to overoxidation by certain substrates also exist within prokaryotes including *E. coli* [Tpx (Baker and Poole, 2003)] and cyanobacteria [*Anabaena* 2-Cys Prx (Pascual, et al., 2010)], suggesting more complexity to the roles of Prxs in these organisms, as well. In yeast, the transcription factors like Pap1 from *Schizosaccharomyces pombe* and Yap1 from *Saccharomyces cerevisiae* do not directly sense peroxides, but instead interact through thiol-disulfide exchange pathways with Prx and Gpx homologues to transmit the oxidation “signal” sensed by these peroxidases (Antelmann and Helmann, 2010, D’Autreaux and Toledano, 2007, Klomsiri, et al., 2010, Veal, et al., 2007). This ability to be reduced by alternative partners requires special features within the “sensor” peroxidases that allow for a kinetic pause in the catalytic cycle after sulfenic acid formation, thereby promoting disulfide bond formation with Cys residues from other proteins. In fact, recent studies of *S. cerevisiae* where all 8 thiol-dependent peroxidases (5 Prxs and 3 sulfur-containing Gpxs) were knocked out demonstrated a profound loss in sensitivity of transcriptional activity toward H₂O₂ in the absence of these proteins, highlighting their dual roles as defense enzymes and signal transduction intermediates (Fomenko, et al., 2011).

The roles played by Prx proteins in cell signaling processes in higher organisms are under intense investigation, and although few would argue that Prxs are not important regulators of cell signaling, the molecular details of how they exert their specific effects and when and where they are important players are the subject of a burgeoning literature (D’Autreaux and Toledano, 2007, Flohé, 2010, Forman, et al., 2010, Hall, et al., 2009, Winterbourn, 2008). For the purpose of this overview, some of the major points of discussion are summarized herein, leaving it to the interested reader to seek further depth to the arguments from the literature.

One major feature of Prx regulation that has gained considerable attention is sensitivity to hyperoxidation caused by turnover of these enzymes in the presence of relatively high levels of peroxide substrates, as discussed above. The idea that sensitivity toward hyperoxidation plays any role in biology is supported by several arguments: (i) sensitivity is an evolved feature which is readily dampened by mutation or modification, (ii) an ATP-dependent enzymatic repair system exists to reverse this inactivating modification, and (iii) hyperoxidation of Prxs can occur within various cell types after stimulation, being caused, for example, by treatment with tumor necrosis factor alpha, 6-hydroxydopamine or expression plasmids for elevating lipoxygenase and cyclooxygenase expression (Cordray, et al., 2007, Lee, et al., 2008, Rabilloud, et al., 2002). In 2003, Karplus and Poole proposed the

floodgate hypothesis, suggesting that this sensitivity coevolved within abundant cellular Prx proteins in concert with the need for localized peroxide production to drive redox-dependent signaling processes (Wood, et al., 2003). By switching “off” the abundant and highly active Prxs around sites of rapid ROS production, like those with activated NADPH oxidase complexes, the oxidants produced could reach higher levels within these foci that could then permit the chemical oxidation of other protein targets involved in promoting or controlling signaling, processes which would normally be much too slow to compete with oxidation of the reactive Prxs. Alternative roles for hyperoxidation have also been proposed, including promotion of a chaperone-like function that accompanies aggregation beyond the level of decamer formation (Jang, et al., 2004, Moon, et al., 2005). The sulfinic (or sulfonic) acid form(s) could also (or instead) act as signals sensed in some way by the cell to elicit appropriate responses. Such a role was suggested by the appearance of aggregates of hyperoxidized PrxII protein within cells that correlated with cell cycle arrest caused by a low grade, continuous exposure to H₂O₂ (Phalen, et al., 2006). After removal of the oxidant, recovery of the non-aggregated forms of PrxII also correlated with the resumption of the normal cell cycle. While the concept that a loss in peroxidase activity caused by Prx hyperoxidation would be sufficient to promote redox-based cell signaling is undergoing considerable debate in the literature (Flohé, 2010, Forman, et al., 2010, Stone and Yang, 2006, Winterbourn, 2008), a new study from the Rhee group uses a parallel argument to describe the redox signaling promoting effects of membrane-localized Prx phosphorylation (Woo, et al., 2010). There is currently a large degree of interest in the idea that Prxs act as transducers of the peroxide signal through redox-regulated interactions with effector proteins (e.g., cAbl, cMyc, JNK1), or by oxidizing Trx proteins which can regulate signaling pathways (e.g., through Ask1 regulation) (Flohé, 2010, Forman, et al., 2010, Winterbourn, 2008). Both the floodgate model and the intermediary sensor role for Prxs are strongly supported by investigations into their roles in yeast, while these models in mammals remain much more speculative at this point (Hall, et al., 2009).

SUMMARY

Peroxiredoxins are abundant cellular antioxidant proteins that help to control intracellular peroxide and peroxynitrite levels. These proteins may also function in regulating hydrogen peroxide signaling in eukaryotes through an evolved sensitivity of some peroxiredoxins towards peroxide-mediated inactivation. The conserved active-site environment activates both the peroxidatic cysteine and the hydroperoxide substrate, supporting a high catalytic efficiency. Oligomeric state may also change with redox state, and can play a role in promoting catalysis.

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Figure 1.

The catalytic cycle of Prx. The three main chemical steps of catalysis are (1) peroxidation, forming the sulfenic acid at the peroxidatic cysteine (S_pOH), (2) resolution which generates a disulfide bond, and (3) recycling by reduction to return the enzyme to its peroxide-reactive state. The cycle requires two conformational states; the fully folded (FF), intact active site is needed for peroxidation, whereas a structural rearrangement to generate the locally unfolded (LU) form is required for resolution. Redox-dependent regulation of Prxs may occur through a second step of peroxide-mediated oxidation of the sulfenic acid when peroxide concentrations are high, and the inactivated enzyme may be rescued through an ATP-dependent reaction catalyzed by sulfiredoxin (Srx). This generic Prx emphasizes the chemistry at the peroxidatic cysteine, with the resolving thiol group ($R'S_rH$) coming either from the Prx itself or another molecule. R'' in the recycling step typically represents disulfide-reducing proteins such as Trx and AhpF.

Figure 2.

Oligomeric interfaces and quaternary structures of Prxs. Dimerization of Prx subunits can occur through two different types of interfaces, the A-type interface (as observed for Tpx and Prx5 subfamily members), or the B-type interface which brings the β sheets together to form an extended sheet (as observed for PrxI/AhpC and Prx6 subfamilies). Some members of the PrxI/AhpC and Prx6 subfamilies also form decameric, or less commonly dodecameric or octameric, structures built from B-type dimers through interaction at their A-type interfaces.

Figure 3.

Interactions around the Prx active site with bound hydroperoxide substrate. The stabilizing hydrogen bonding interactions (dotted lines) between key atoms from the backbone and the four conserved residues, and with the ROOH substrate, are indicated. The geometry of the active site is ideal for stabilizing the larger distance between O_A and O_B atoms as the bond is broken. Adapted from (Hall, et al., 2010).

Table I
Summary of the structural and bioinformatic analyses of Prx subfamilies

Subfamily	Phylogenetic Distribution	Structural distinctions relative to Prx core fold ^a	Oligomeric states and interfaces	Typical Location and Conservation of C _r (When Present) ^a
AhpC-Prx1 ^b	Archae, Bacteria, Plants and Other Eukaryotes	Extended C-terminus	B-type dimers, form decamers through A-interface ^c	C-terminus of another subunit (>96%) ^d
Prx6 ^e	Archae, Bacteria, Plants and Other Eukaryotes	Longer extended C-terminus	B-type dimers, may form decamers through A-interface	Rare
Prx5 ^f	Bacteria, Plants and Other Eukaryotes	α -Aneurysm in helix α 2, ~16% fused with Grx domain	A-type dimers	Helix α 5 (~17%) ^g
Tpx ^h	Bacteria	N-terminal β -hairpin	A-type dimers	Helix α 3 (>96%) ^g
BCP/PrxQ ⁱ	Archae, Bacteria, Plants and Fungi	Longer helix α 5	Typically monomeric, may form A-type dimers	Helix α 2 (~55%) or α 3 (~7%) ^g
AhpE	Bacteria ^j	Extended loop at N-terminus	A-type dimer(?) ^j	Uncertain ^j

^aStructural designations as in (Hall, et al., 2011). If no C_r is present, resolving thiol must come from another protein or small molecule.

^b AhpC/Prx1 is also known as the “typical 2-Cys” Prxs and includes *Salmonella typhimurium* AhpC, *Homo sapiens* Prx1 through PrxIV trypanothione peroxidases, *Arabidopsis thaliana* 2-Cys Prx, barley Bas1, and *Saccharomyces cerevisiae* TSA1 and TSA2.

^cOctamers and dodecamers have also been observed (Hall, et al., 2011).

^dThe C_r is near the C-terminus of the partner subunit within the homodimer; upon oxidation, intersubunit disulfide forms between the C_p and the C_r of the two chains.

^eThe Prx6 subfamily (frequently referred to as the “1-Cys” group) includes *H. sapiens* PrxVI, *Arenicola marina* PRDX6, *A. thaliana* 1-Cys Prx and *S. cerevisiae* mitochondrial Prx1.

^fThe Prx5 subfamily includes *H. sapiens* PrxV, *Populus trichocarpa* PrxD, the plant type II Prxs, mammalian Prx 5, and a group of bacterial Grx-Prx5 fusion proteins.

^gIntrasubunit disulfide formed in oxidized protein (so-called “atypical” 2-Cys Prxs).

^hThe Tpx subfamily includes bacterial proteins (e.g. from *E. coli*, *Streptococcus pneumoniae* and *Helicobacter pylori*) named thiol peroxidase, p20, and scavengase.

ⁱThe BCP/PrxQ group includes *Escherichia coli* bacterioferritin comigratory protein and plant chloroplast PrxQ.

^jThe canonical AhpE from *Mycobacterium tuberculosis* contains no C_r and is dimeric; however, >50% of sequences include a potential C_r in α 2, similar to *E. coli* BCP. Distribution appears restricted to the order *Actinomycetales*.

Table 2
Summary of measured pK_a values for the C_p of Prxs

Protein name	Biological origin	pK _a of C _p	Reference
Tsa1	<i>Saccharomyces cerevisiae</i>	5.4	(Ogusucu, et al., 2007)
Tsa2	<i>Saccharomyces cerevisiae</i>	6.3	(Ogusucu, et al., 2007)
PrxV	<i>Homo sapiens</i>	5.2	(Trujillo, et al., 2007)
AhpC	<i>Salmonella typhimurium</i>	5.9	(Nelson, et al., 2008)
PrxII	<i>Homo sapiens</i>	5.3	(Manta, et al., 2009)
AhpE	<i>Mycobacterium tuberculosis</i>	5.2	(Hugo, et al., 2009)
PrxQ	<i>Xylella fastidiosa</i>	6.2	(Horta, et al., 2010)