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Review

The oligomeric conformation of peroxiredoxins links redox state to function

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ABSTRACT

Protein–protein associations, i.e. formation of permanent or transient protein complexes, are essential for protein functionality and regulation within the cellular context. Peroxiredoxins (Prx) undergo major redox-dependent conformational changes and the dynamics are linked to functional switches. While a large number of investigations have addressed the principles and functions of Prx oligomerization, understanding of the diverse in vivo roles of this conserved redox-dependent feature of Prx is slowly emerging. The review summarizes studies on Prx oligomerization, its tight connection to the redox state, and the knowledge and hypotheses on its physiological function in the cell as peroxidase, chaperone, binding partner, enzyme activator and/or redox sensor.

1. Introduction

Peroxiredoxins (Prx) function as thiol peroxidases independent of cofactors such as metal ions or prosthetic groups. Peroxide reduction takes place by an oxidation of the peroxidatic thiol (C_P) residue that acts on the O–O bond of the peroxide (Fig. 1). In a second step the generated sulfenic acid derivative of the C_P reacts with an adjacent thiol (resolving cysteine C_R) forming an intra- or intermolecular disulfide bond. In a third step the dithiol state is re-reduced via electron donors such as thioredoxin reductase C, thioredoxin, glutaredoxin, glutathione, cyclophilin, ascorbate or DTT [1].

Disulfide bridge formation depends on a slow structural rearrangement of Prx protein to bring closer both cysteinyl residues [2,3]. In some cases, the sulfenic acid group is subjected to glutathionylation, hyperoxidation to sulfinic acid (SOOH) or S-nitrosylation [4]. Hyperoxidation of C_P to sulfinic acid was thought to be an irreversible modification until the discovery of the sulfinic acid reductase sulfiredoxin (Srx) [5] (Fig. 1D). The floodgate theory suggests that H_2O_2 is kept low by Prx activity in normal cell, while Prx inhibition by hyperoxidation enables H_2O_2 signaling. Although Prxs are present in all studied organisms, marked differences exist between them. For example, only eukaryotic Prxs are highly sensitive to hyperoxidation probably due to the presence of the GGLG and YF motifs, absent from bacterial AhpC, which facilitates the conformational switch needed for disulfide formation in the catalytic cycle [6].

Prxs are classified by number of cysteines implicated in catalysis (1-CysPrx and 2-CysPrx), by formation of either inter- or intramolecular disulfide bonds (typical and atypical) or by oligomerization properties as discussed below (Fig. 1A–C). Non-uniform classification and use of diverse trivial names for the same Prx protein impede simple text mining and complete searches. Table 1 and Fig. 1 summarize the catalytic mechanisms, the regenerators, the biological sources, trivial names and abbreviations of those Prx addressed in the review. Since the first observation of doughnut-shaped Prx-oligomers by transmission electron microscopy, the dynamic equilibrium between assembly and disassembly has been extensively studied and shown to depend on ionic strength, protein concentration, pH, phosphorylation and first of all on the redox state.

1.1. Structural basis for dimer and oligomer formation

Except for the monomeric Prx of the C-type (bacterioferritin comigratory protein and PrxQ), Prxs are obligate dimers [7]. These dimers can form intermolecular disulfide bonds or remain reduced.

Abbreviations: HMW, high molecular weight; ITC, isothermal titration microcalorimetry; LMW, low molecular weight; Prx, peroxiredoxin; SEC, size exclusion chromatography.

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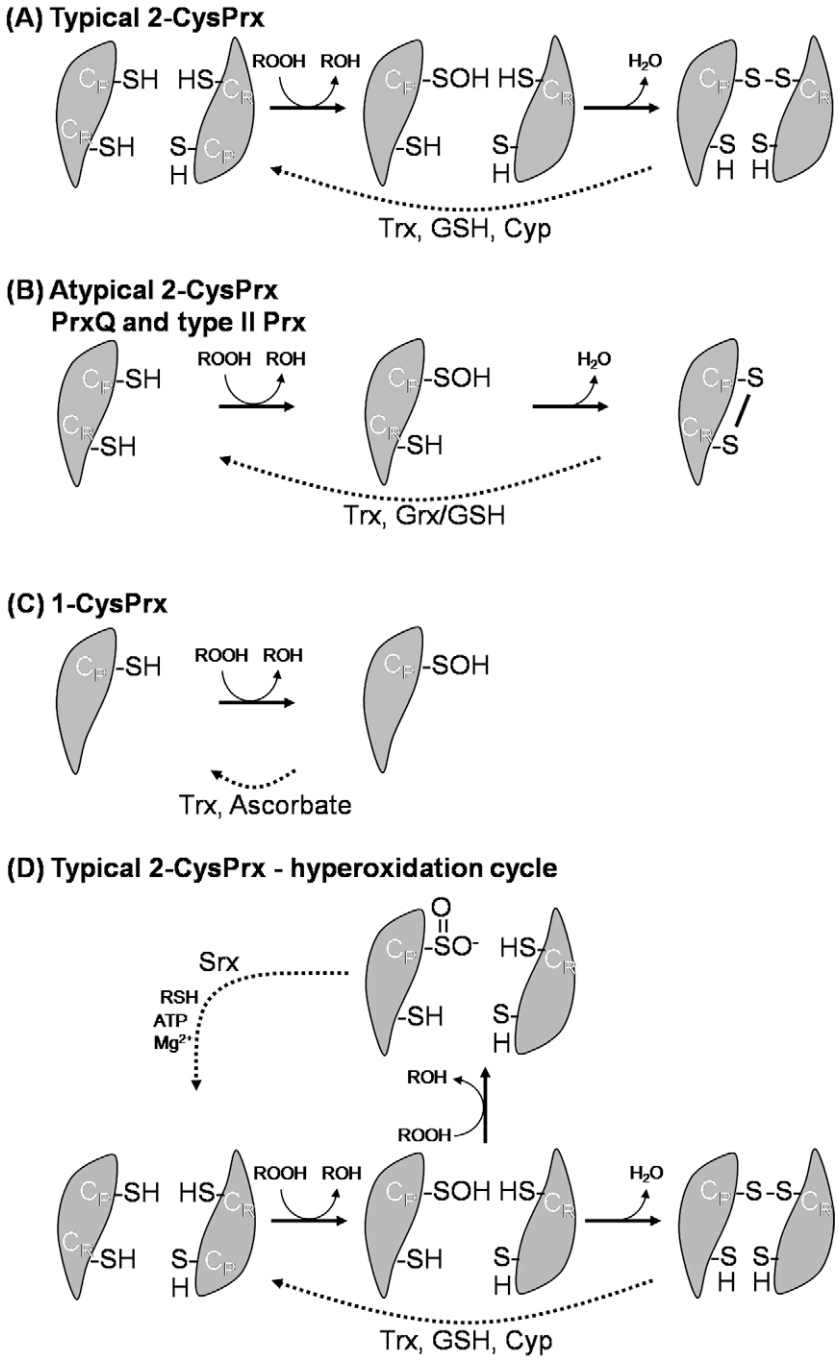

Fig. 1. Catalytic mechanism of the three types of peroxiredoxins. The peroxidatic catalytic Cys residue C_P primarily reacts with the peroxide substrate. In typical 2-CysPrx, C_P reacts with a resolving Cys residue C_R on the second subunit of the dimer (A), while in atypical 2-CysPrx the resolving Prx is located on the same polypeptide chain (B) either in close vicinity (PrxQ) or more distantly (TypeII Prx, Prdx5). The sulfenic acid derivative of 1-CysPrx is directly regenerated by an electron donor to the thiol form (C). Cyp: cyclophilin; Grx: glutaredoxin; GSH: glutathione; ROOH: peroxide; RSH: electron donor; Srx: sulfiredoxin; Trx: thioredoxin.

The interfaces between the dimer subunits are categorized as parallel (A, B and F homodimers) or perpendicular (D and E homodimers), the interface between dimers as perpendicular (A and F decamers) according to the orientation of the central β-sheet [3,7]. The parallel orientation appears more stable than the perpendicular one [7–9]. With C_p and C_R reduced, the structure of 2-CysPrx is fully-folded. The reaction with the peroxide substrate oxidizes the C_p to sulfenic acid which is protected in the active site pocket by the first turn of the helix α_2. To enable the attack and reduction of C_p a partial unfolding of the structure by movement of helix α_2 is needed to form the 'C_p-loop' [2]. This intermediate structure is partial-unfolded in dynamic equilibrium with the fully-folded Prx. Finally the disulfide bridge is formed and the oxidized Prx is locked in the partially-unfolded state. The conformational change weakens the perpendicular interface and in many cases the decamer disassembles to dimers. The C_P-loop acts as molecular switch in oligomerization. Site-directed mutation of Thr^{77} at the dimer–dimer interface (variants T77I and T77D) disrupts the decamer while the variant T77V stabilizes the decamer [10]. This implies that the decameric form and the fully-folded active site conformation are linked. Cyclin-dependent protein kinases phosphorylate Thr^{90} in human PrxI and inhibit its activity by about 80% [11], an effect which was attributed to decamer breakdown favored by electrostatic repulsion of two adjacent negatively charged

Table 1
Compilation of peroxiredoxins addressed in the text for its specific features.

Prx type	Specific name	Synonyms	Species
Typical 2-CysPrx	AhpC	Alkylhydroperoxide reductase C	*Amphibacillus xylanus*
			Helicobacter pylori
			Mycobacterium tuberculosis
			Salmonella typhimurium
	At-2-CysPrx	Bas1	*Arabidopsis thaliana*
	Hv-2-CysPrx		*Hordeum vulgare*
	Ps-2-CysPrx		*Pisum sativum*
	Prdx1	Hs-Prx1	*Homo sapiens*
		Heme binding protein 23 (HBP23)	*Rattus norvegicus*
	Prdx2	Thioredoxin peroxidase	*Homo sapiens*
		TPx-B	*Bos taurus*
		Natural killer enhancing factor B (NKEF-B)	
		Calpromotin	
		Torin	
	Prdx3	Prx-3	*Homo sapiens*
			Other vertebrates
	Pf-Prx2		*Plasmodium falciparum*
	TryP	Tryparedoxin peroxidase	*Trypanosomas brucei*
1-CysPrx	Ap-1-CysPrx		*Aeropyrum pernix*
	Mt-AhpE	Alkylhydroperoxide reductase E	*Mycobacterium tuberculosis*
	Prdx-6		*Homo sapiens*
	Prx6		*Arenicola marina*
	Se-PrxQ-A1		*Synechococcus elongatus*
	Tg-Prx2		*Toxoplasma gondii*
Atypical 2-CysPrx	Prdx5	Hs-Prx5	*Homo sapiens*
	PrxII-F	Plant mitochondrial type II PrxF	*Pisum sativum*
	Prx5		*Haemophilus influenza*
	PrxQ	Bacterioferritin comigratory protein	Bacteria
	Prx-typeC		

phosphate groups [12]. It should be noted that size exclusion chromatography (SEC) analysis of the oligomeric state of His-tagged Prx3 indicates stabilization of the dodecamers indicating that the dimer–decamer equilibrium of His-tagged Prx might be biased. This needs to be kept in mind when working with tagged recombinant Prx protein.

2. The conformational dynamics of typical 2-CysPrx

The typical 2-CysPrx adopts four different conformation states which are linked to switches in function (Fig. 2). The reduced and hyperoxidized dimers strongly tend to form decamers or dodecamers [12,13], while the oxidized form is preferentially present as dimer. In addition, part of the reduced protein also exists as dimer. Isothermal titration microcalorimetry (ITC) of 2-CysPrx from plant to human sources reveals highly similar critical concentrations (K_d) for oligomer formation ranging between 1 and 2 μM below which the oligomer disassembles [14]. The critical concentration for the dimer–oligomer transition is not affected by the presence of fused tags such as 6xHis, at least not to a significant extent [14]. This thermodynamic study also proved that oligomerization of reduced 2-CysPrx in the critical concentration range is a

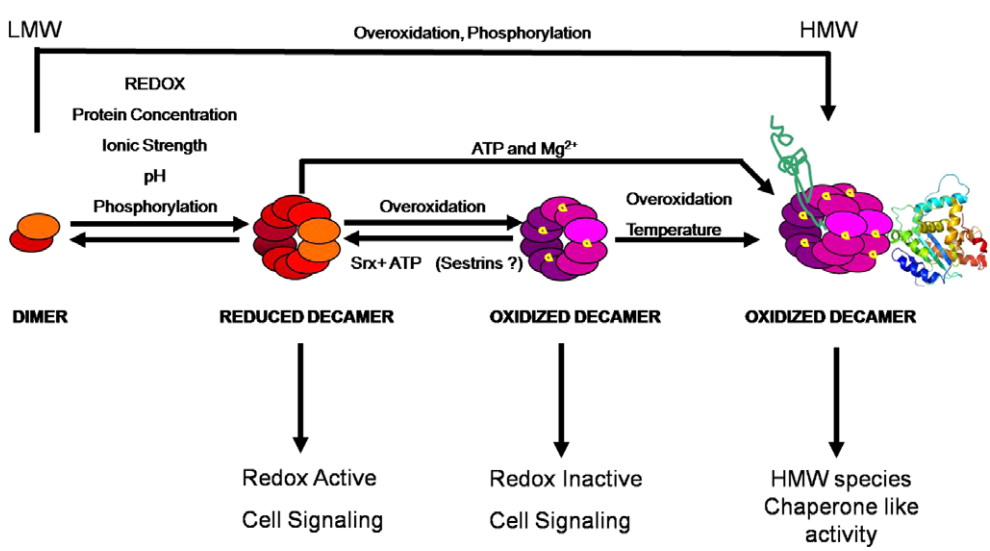

Fig. 2. Integrative model of 2-CysPrx oligomerization and function. In response to different factors, especially under reducing conditions, dimers oligomerize to hexa-, octa-, decamers or higher order aggregates with peroxidase function. Oxidation leads to the breakdown of the decamers, whereas hyperoxidation stabilizes the oligomer. Re-reduction of hyperoxidized Prx is achieved by Srx. If hyperoxidation persists Prxs can convert to HMW species with chaperone like activity.

highly cooperative process excluding the existence of partially assembled complexes to any significant extent.

For plants, SEC with recombinant 2-CysPrx from *pea* (lacking His-tag) [15] and barley [16,17] reveals a preference for decameric state under reducing conditions, high phosphate concentration, high ionic strength and low pH. The dimer–decamer transition occurs while lowering the pH from 8.0 to 7.5. This indicates the presence of a protonatable amino acid residue that supports decamer formation. As shown by ITC dilution experiments, oligomerization of typical 2-CysPrx from *Arabidopsis thaliana*, *Pisum sativum* and *Homo sapiens* is triggered above a critical concentration $1 < K_d < 2$ μM [14]. Thus, in light of the chloroplast 2-CysPrx concentration above 60 μM [16] reduced 2-CysPrx appears to exist as decamer. In a converse manner, dimeric oxidized and decameric hyperoxidized Prx does not show heat changes upon dilution showing that the oxidized form does not aggregate and the hyperoxidized oligomer not dissociate. This confirms the distinctness of the four conformational states.

The $\text{dimer}_{red} \rightarrow$ (do-)decamer_{red}-interconversion is suggested to be dynamic with continuous formation and disassembly of the oligomer. Under oxidizing conditions the decamer breaks down releasing free dimers with intermolecular disulfide bonds that are efficiently regenerated by redox proteins, again a dynamic cycle. However, hyperoxidation may freeze the decamer potentially exerting other functions as chaperone (see below). Complementary assays mixing 2-CysPrxs from different species revealed that the dimer–dimer interfaces involved in cooperative binding have differentiated during evolution [14].

2.1. High molecular mass assemblies

Decamers may associate to higher order aggregates [high molecular weight (HMW) form]. The dodecahedron formation in vitro (12 decamers) is favored by hyperoxidation, polyethyleneglycol and ammonium molybdate, and its occurrence in erythrocytes in vivo is hypothesized [18]. Other peculiar crystal structures such as two-ring catenanes of bovine Prx3 comprised of two interlocked dodecamers [19] also seen in TEM with a frequency of 3–5% [20] may result from very high protein concentrations in vitro without physiological significance. After re-dissolving catenane-containing crystals of C186S Prx3, TEM analysis revealed that the population largely consisted of single toroidal structures suggesting that catenane formation is a dynamic and stochastic process. Based on in vivo and in vitro experiments Jang et al. described the dual function of yeast PrxI as peroxidase and chaperone and linked it to its quaternary structure which changed between 40 and >1000 kDa [21]. Hyperoxidation induces oligomerization from low molecular weight (LMW) to HMW species with two dodecamers that adopt chaperone function [22]. Truncation of the C-terminal loop in *Schizosaccharomyces pombe* Prx generated a hyperoxidation-resistant variant underlining the significance of this structural element for susceptibility to hyperoxidation [23]. Phosphorylation of Thr^{90} eases the conversion from LMW peroxidase to HMW chaperone [24]. Phosphorylation induces the exposure of hydrophobic domains involved in HMW complex formation. Production of mutant K90E protein mimics Thr^{90} phosphorylation which underlines the importance of this residue.

2.2. Oligomerization of procaryotic Prx

The prokaryotic typical 2-CysPrx known as AhpC is one of the best studied Prx and presents features distinct from its eukaryotic homologues. AhpC lacks the C-terminal YF motif and has a highly flexible C-terminal region. This flexibility facilitates formation of the intermolecular disulfide bond and suppresses hyperoxidation, thereby rendering AhpC more resistant to oxidative inactivation. The AhpC from *Amphibacillus xylanus* crystallizes as decamer [25] and separates as decamer during SEC even under oxidizing conditions. Dynamic light scattering (DLS) relates oligomerization with ionic strength with little influence of salt type [25]. Salt concentrations above 300 mM favor oligomerization and stimulate peroxidase activity. Oligomerization is proposed to induce favorable changes in the active site that increase catalytic efficiency.

During analytical ultracentrifugation reduced *Salmonella typhimurium* St-AhpC sediments as decamer at all tested protein concentrations while the oxidized enzyme is a dimer at low, a decamer at high and a mixture of dimers and decamers at intermediate concentrations, respectively. As with other typical 2-CysPrx St-AhpC oligomerization is redox sensitive with reduction favoring decamerization. Crystallized AhpC exhibits a decameric arrangement both in reduced and oxidized state owing to the high protein concentration used for crystallization [2]. These authors suggested the formation of a weakly associated decamer as metastable intermediate. Structural comparison of the tightly bound reduced decamers of Hs-Prdx2 [8] or TryP [26] and the oxidized dimer of *Rattus norvegicus* Prx1 [27] with the oxidized weak decamer of AhpC suggests that the C_P-loop (DFTFVCPTE) functions as molecular switch controlling decamerization.

Mt-AhpC from *Mycobacterium tuberculosis* separates as HMW complex of 10–12 subunits during SEC. In contrast to other 2-CysPrxs, the oligomers dissociate at high salt concentrations [28]. Subsequent structure analysis of the oxidized protein deconvoluted a dodecamer of donut-like shape consisting of six dimers despite the free dimer detected during SEC.

A new mechanism involving three cysteine residues ($Cys^{61\text{-}174\text{-}176}$) in the catalytic cycle was proposed for the interaction between AhpC and AhpD [29]. Hp-AhpC from *Helicobacter pylori* crystallizes as toroid-shaped complex [$(\alpha_2)_5$ decamer] [30]. At relatively high ionic strength and under reducing conditions the equilibrium shifts towards the decamers, whereas the dimer dominates at low ionic strength. Hp-AhpC sequence and structure resemble mammalian Prx more than eubacterial Prx. Hp-AhpC function switches from peroxidase to chaperone concomitant with a change from LMW to HMW state [31]. Albeit the oxidation state of the cysteines was not addressed, this report poses a new question because AhpC does not possess the YF-motif, so in theory it should not be prone to hyperoxidation. In addition, the lack of sulfiredoxin in *H. pylori* could imply the irreversibility of eventually occurring hyperoxidized forms. In summary, the results from different bacterial 2-CysPrx confirm the wide occurrence of the dimer_{ox}–oligomer_{red} transition despite some differences such as the HMW aggregate formation in *Helicobacter* or the lack of dimerization e.g. in *Synechococcus elongatus* PCC 7942 [32].

2.3. Functional significance of the conformational switch

The reviewed data indicate that the ability of typical 2-CysPrx of eukaryotic as well as prokaryotic origin to perform conformational switches is highly conserved among species. The degree of evolutionary conservation with species-specific variation implies that the transition between reduced dimer, reduced oligomer, oxidized dimer and hyperoxidized HMW assemblies relates to function, i.e. as peroxidase, chaperone, binding partner, enzyme activator and/or redox sensor. During the standard cycling between reduced decamer and oxidized dimer, the reduced decamer appears to increase the peroxidase activity while the disassembly of the oligomer upon oxidation may ease regeneration by the electron donor [11].

Vertebrate Prx2 is the third most abundant protein of erythrocytes. Its equilibrium between dimer and oligomer shifts to oligomer under reducing conditions [8]. Prx2 associates with the plasma membrane in a calcium- and conformation-dependent

manner and activates the charybdotoxin-sensitive and calcium-dependent potassium channel thereby triggering K-efflux [33]. Membrane attachment involves a strong interaction between calpromotin and the membrane protein 7.2b named stomatin [34]. The human Prx2 decamer disassembles both at pH > 7.8 and urea concentrations higher than 2.5 M [35]. Amino acid groups that titrate at this physiological pH could control the oligomerization process. Calcium-dependent attachment of Prx2 to the inner surface of the erythrocyte membrane correlates with a decrease of the cytosolic pH from 7.29 to 7.14 in patients with sickle cell disease. Based on the decameric structure model, attachment of Prx2 to membrane proteins appears to be favored by hydrophobic patches at the outer face of the torus. This mechanism might link redox sensing with osmoregulation of erythrozytes.

The oligomeric state controls the switch from peroxidase to chaperone function of human Prx2 [36]. Truncation of the C-terminal 'YF' motif obstructs formation of the HMW state. As a consequence the oxidation-sensitive Prx2 converts to a hyperoxidation-resistant form. In addition a specific cleavage of Prx2 at the C-terminal domain may regulate the switch in structure and function.

Although human Prx1 and Prx2 share 91% amino acid sequence similarity, Prx1 contains an additional Cys^{83} at the dimer–dimer interface [37]. X-ray crystallography resolved the human Prx1 as a decamer in which the five dimer–dimer interfaces involve Cys^{83} disulfide bridges between the dimers suggesting that Prx1 decamer is locked by disulfide bonds. Gel filtration analysis and mass spectrometry revealed that Prx2 as well as mutant Prx1C83S exist as dimers, whereas Prx1 forms a decamer. Thus Prx1 might preferentially function as chaperone, while Prx2 as peroxidase. In a converse manner, the decameric crystal structure of mutant C52S of Prx1 (rat HBP23 heme-binding protein 23) is not or only partly stabilized by Cys^{83}–Cys^{83} bonds in the dimer–dimer interface [38] since only one out of the five dimer–dimer interfaces is implicated in disulfide bond formation. Low Prx1 concentration or high ionic strength foster dissociation of the decamer, whereas high protein concentration as well as DTT increase the decameric fraction suggesting that decamer formation is independent on disulfide bridges. The authors suggested that the variant C83S of the rat Prx1 is incapable of forming decamers not because of its implication in disulfide bridges at the interface, but because the replacement of a sulfur atom with oxygen facilitates decamer dissociation.

The HMW form is favored by hydrogen peroxide, high temperature and oxygen and reveals a strong chaperone and no or very low peroxidase activity, whereas the LMW form primarily exhibits peroxidase activity (Fig. 2) [21]. Similar dependencies have been described for the chaperone HSP33 [39]. Thus HMW-Prx protected citrate synthase from aggregation during chemically or heat induced denaturation. The HMW to LMW conversion seems to be linked to the re-reduction by sulfiredoxin and the multimerization could involve thioredoxin. In addition, it was denoted that hyperoxidation of Cys^{47} could serve as an efficient ROS sensor in vivo and might be implicated in the dynamic changes in quaternary structure.

In plants the redox and pH-dependency of 2-CysPrx oligomerization links changes in chloroplast redox- and pH-milieu to suborganellar distribution and function of 2-CysPrx. Both reduced and hyperoxidized barley 2-CysPrx associate with thylakoids [17]. During the light–dark transition but also in dependence of the balance between excitation pressure and energy consumption in photosynthetic metabolism the chloroplast redox milieu shifts between a more oxidized and reduced state and the pH between 7.0 and 8.0 [15], both with implications for 2-CysPrx conformation. Expression of 2-CysPrx early during leaf development [40,41], impairment of photosynthesis in antisense plants with decreased 2-CysPrx amounts [42] and redox-dependent attachment to the thylakoid [16] suggest that plant 2-CysPrx acts as redox sensory system that modifies photosynthetic assembly or function. Oligomerized rapeseed 2-CysPrx activates chloroplast fructose-1,6-bisphosphatase (FBPase) [43]. In addition to the generally accepted thioredoxin-dependent activation by reduction, the redox-independent activation of FBPase by 2-CysPrx presents a novel mechanism that allows to modulate Calvin cycle activity under oxidizing conditions. Mg^{2+} and ATP reversibly inhibit rapeseed 2-CysPrx peroxidase activity and converts decamers to HMW complexes with masses similar to the dodecahedron described for Prx2 [44]. Strikingly, increasing amounts of Mg^{2+} decrease the chaperone activity and enhance oligomerization. The phosphorylation is assigned to the hyperoxidized C_R residue forming a sulfinic/sulfonic-phosphoryl anhydride. It was suggested that C_R constitutes a dual sensor able to perceive changes in cellular redox and energy status.

3. Atypical 2-CysPrx oligomerization

Increasing evidence indicates that atypical 2-CysPrxs also undergo protein–protein interactions with functional implications. Although oligomerization occurs within the group of atypical Prxs, the lower polymerization degree (dimers, tetramers and hexamers) in comparison with typical 2-CysPrxs (decamers and HMW species) seems to be related to a differentiation in both reaction mechanism and physiological function. Dimerization of atypical Prxs is based on A-type interfaces [3,9]. In addition to dimerization, the presence of HMW species has been established recently. Human Prx5 is the prototype for atypical 2-CysPrx. Its crystal structure shows an intermolecular disulfide between C_P and C_R of different dimers in addition to intramolecular disulfide bridges. Bridge formation depends on an unfolding of the first half of helix α5 and C_P-loop [45]. The detection of intramolecular disulfide bonds in the structure [46] confirms the previously proposed reaction mechanism [45] where unwinding of the N-terminal part of the a2 helix containing C_P in the reduced form allows for the intramolecular disulfide formation with C_R. Co-crystallization between Prx5 and Trx2 supports a structural arrangement where about 15 Prx5 define a cavity occupied by one Trx2. If this model is verified the concept of single Prx5 dimers needs to be modified.

Pea mitochondrial PrxIIF occurs as hexamer indicating a dynamic dimer–hexamer equilibrium for an atypical 2-CysPrx [47]. This finding urges future studies on possible chaperone function similar to those described for typical Prxs. Unlike typical 2-CysPrx, PrxIIF oligomers dissociate under reducing conditions and after mutation of C_P while oxidation and high pH trigger hexamer formation. Electrophoresis of the hexamer revealed the existence of inter- and intramolecular disulfide bonds. Hexamers were also found in the unit cell of PrxIIF crystals [48]. Additional results indicate that a regular Trx-o/PrxIIF-assembly increases the catalytic activity of this mitochondrion-localized type II Prx.

Another structural peculiarity is presented by the redox-independent tetramer of *Haemophilus influenza* hybrid Prx5 which possesses a Prx and a Grx domain [49]. The crystal structure, analytical ultracentrifugation, SEC, LDS and activity measurements link complex formation with electron transfer efficiency from the Grx domain to the Prx domain. Some other bacterial thioredoxin-dependent thiol peroxidases (Tpx) also belong to the atypical 2-CysPrx group. Several crystal structures reveal dimers under different redox states [50–52]. However, in depth analysis of occurrence and functional significance of atypical Prx assemblies are scarce and need to be performed in the future.

4. 1-CysPrx oligomerization

1-CysPrx has been much less studied and the information compiled from the literature does not offer clear insights into the

physiological function of 1-CysPrx oligomerization. The heterogeneous group of 1-CysPrx comprises a variety of proteins with one or more Cys and with different reaction mechanisms. The human recombinant 1-CysPrx structure was resolved as a dimer [53] while monomers and dimers have been observed in solution using gel filtration [54]. The formation of 1-CysPrx dimers is exclusively ascribed to non-covalent interactions. The so called 1-CysPrx from *Toxoplasma gondii* (Tg-Prx2) is special among the 1-CysPrx group both in terms of oligomerization and enzyme mechanism. Tg-Prx2 possesses 5 cysteine residues, but has been classified as 1-CysPrx based on sequence relatedness. However, the C_P^{47} forms an intermolecular disulfide bridge with C-terminal C_R^{209} from a second subunit employing a similar reaction mechanism as described for typical 2-CysPrx [55]. Based on structural prediction, the formation of this disulfide bond involves an unfolding of the α-helix and a conformational switch. Gel filtration and SDS–PAGE confirmed the existence of non-covalent dimers. Addition of H_2O_2 or incubation at 37 °C increased the fraction of oxidized dimer possessing an intermolecular disulfide bridge. Tg-Prx2 treated with H_2O_2 and 1.7 M NaCl, respectively, formed dimers, tetramers and hexamers. This behavior resembles that of atypical pea mitochondrial PrxIIF. In both cases, the addition of H_2O_2 induces the hexamerization. Although the authors did not address potential roles of the Tg-Prx2 hexamers, it is tempting to speculate that they feature an activity similar to that described for PrxIIF oligomers.

Similar aggregation versatility was described for archaeal 1-CysPrx from *Aeropyrum pernix* [56]. Electron microscopy, gel filtration and analytical ultracentrifugation showed the presence of monomers, octamers and hexadecamers (twofold toroid-shaped oligomers). Although oligomerization did not involve intersubunit-disulfide linkages site-directed mutagenesis proved that Cys^{50} is essential for hexadecamer formation. The crystal structure revealed 10 monomers arranged in a toroid shaped decamer [57]. Ap-Prx possesses 3 cysteinyl residues that form intermolecular disulfide bridges between Cys^{50} and Cys^{213} in the normal reaction mechanism in the fully-folded conformation or intramolecular disulfide bonds between Cys^{207} and Cys^{213} in the locally unfolded state at very high H_2O_2 concentration. The authors proposed that archaeal Prxs containing the Cys^{207} could be classified mechanistically as 3-CysPrx.

1-CysPrx can also be found within the Prx6 subfamily. Prx6 from the marine annelid *Arenicola marina* has a high amino acid sequence identity with human 1-CysPrx but with five cysteines, two of which function as peroxidatic and resolving cysteines, similar to typical 2-CysPrx [58]. Despite this analogy, Prx6 cannot form toroid-shaped decamers possibly due to the structure of region IV corresponding with the loop between the two strands β6 and β7 [2]. In the crystal structure of the C45S variant, dimer pairs form tetramers. The inability to form decamers could indicate that this 1-CysPrx functions as antioxidant rather than as molecular chaperone despite its 2-CysPrx-like enzymatic mechanism. Recombinant rat Prx6 shows monomers, dimers and higher oligomers using native reducing electrophoresis [59]. Other biophysical approaches will be needed to assess the significance of these observations.

A single cysteinyl residue defines AhpE from *M. tuberculosis* as 1-CysPrx, but its sequence and atomic structure relate it to typical 2-CysPrx [60]. Mt-AhpE lacks the C-terminal domain of typical 2-CysPrxs that carries the resolving cysteine. Crystal packing reveals the existence of both, dimers and octamers with a dimerization interface similar to the human Prx5. Both forms are also observed in solution by SEC and light scattering analysis. The dual role as chaperone and peroxidase still needs to be assessed for 1-CysPrx oligomers in a comprehensive manner.

Recently, studies performed with type-D 1-CysPrxs show that glutathionylation of the C_P provokes unwinding of the α_2 helix and disassembly of the non-covalent perpendicular-type homodimer which can be recovered by addition of DTT [61]. This quaternary structure transition between homodimer and monomer mimics the decamer–dimer switch described for typical 2-CysPrx.

A recent 1-CysPrx oligomerization study dealt with PrxQ-A1 from *S. elongatus* which separated as monomer during SEC [32].

1-CysPrx forms heterocomplexes with other proteins. Glutathione *S*-transferase (πGST) and 1-CysPrx form a stochiometric 50 kDa dimer that has been characterized by light scattering [62]. Fluorescence titrations of digested purified fragments of πGST and 1-CysPrx identified hydrophobic interactions as major force implicated in heterodimer stabilization. Another case of heterocomplex has been reported for Prx6 and Surfactant Protein A based on co-immunoprecipitation, gel filtration, affinity chromatography and cross-linking [54]. The recent identification of ascorbate as electron donor to plant 1-CysPrx opens new perspectives as to the interrelation between the thiol-disulfide redox network and ascorbate as another important redox component of the cell [63].

While the group of typical 2-CysPrx is highly uniform and structurally and functionally highly conserved, both the atypical 2-CysPrx and the 1-CysPrx show structural and functional variation that is still insufficiently addressed. In addition there is a need for a uniform nomenclature.

5. Outlook

Although oligomerization is now a widely accepted feature of Prx, its in vivo dynamics is poorly and its physiological significance only partly understood. The employment of biophysical techniques such as fluorescence resonance energy transfer or bioluminescence energy transfer may provide insight into the in vivo dynamics of oligomerization. The switching between the diverse Prx functions, i.e. as peroxidase, redox sensor, chaperone and versatile interaction partner, needs to be addressed in detail both in vitro and in vivo. Generation of transgenic organisms overexpressing Prx arrested in specific states offers a promising approach to tackle this issue, e.g. by substituting C_P by a negatively charged amino acid mimicking the hyperoxidized form and by using the Cys → Ser-variant to simulate the reduced form. Comprehensive searches for interacting partners by methods such as yeast-2-hybrid screening and pull-down assays will also help to address the function of Prx as redox-dependent interactors. As described for Hs-Prx2 and At-2-CysPrx the multiplicity of functions and interactions indicate that Prx function as regulatory hub proteins linking cell redox-state but also e.g. pH to the regulation of cell processes. In terms of biotechnology, the dodecahedron cage could be used as a protein nanocage for the transport of enclosed vectors or the creation of magnetic nanoparticles [18,64].

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References

[1] Perez-Ruiz, J.M., Spínola, M.C., Kirchsteiger, K., Moreno, J., Sahrawy, M. and Cejudo, F.J. (2006) NTRC is a high efficiency redox system for chloroplast protection against oxidative damage. Plant Cell 18, 2356–2368.
[2] Wood, Z.A., Poole, L.B., Hantgan, R.R. and Karplus, A. (2002) Dimers to doughnuts: redox-sensitive oligomerization of 2-Cysteine peroxiredoxins. Biochemistry 41, 493–5504.
[3] Echalier, A., Trivelli, X., Corbier, C., Rouhier, N., Walker, O., Tsan, P., Jacquot, J.P., Aubry, A., Krimm, I. and Lancelin, J.M. (2005) Crystal structure and resolution NMR dynamics of a D (type II) peroxiredoxin glutaredoxin and thioredoxin dependent: a new insight into the peroxiredoxin oligomerism. Biochemistry 44, 1755. 1767.
[4] Romero-Puertas, M.C., Laxa, M., Mattè, A., Zaninotto, F., Finkemeier, I., Jones, A.M., Perazzolli, M., Bandelle, E., Dietz, K.J. and Delledonne, M. (2007) S-

Nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell 19, 4120–4130.
[5] Biteau, B., Labarre, J. and Toledano, M.B. (2003) ATP-dependent reduction of cysteine–sulphinic acid by *S. cerevisiae* sulphiredoxin. Nature 425, 980–984.
[6] Wood, Z.A., Poole, L.B. and Karplus, P.A. (2003) Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. Science 300, 650–653.
[7] Noguera-Mazon, V., Krimm, I., Walker, O. and Lancelin, J.M. (2006) Protein–protein interactions within peroxiredoxin systems. Photosynth. Res. 89, 277–290.
[8] Schroder, E., Littlechild, J.A., Lebedev, A.A., Errington, N., Vagin, A.A. and Isupov, M.N. (2000) Crystal structure of decameric 2-Cys peroxiredoxin from human erythrocytes at 1.7 Å resolution. Struct. Fold Des. 8, 605–615.
[9] Karplus, P.A. and Hall, A. (2007) Structural survey of the peroxiredoxins. Subcell Biochem. 44, 41–60.
[10] Parsonage, D., Youngblood, D.S., Sarma, G.N., Wood, Z.A., Karplus, P.A. and Poole, L.B. (2005) Analysis of the link between enzymatic activity and oligomeric state in AhpC, a bacterial peroxiredoxin. Biochemistry 44, 10583–10592.
[11] Chang, T.S., Jeong, W., Choi, S.Y., Yu, S., Kang, S.W. and Rhee, S.G. (2002) Regulation of peroxiredoxin I activity by Cde2-mediated phosphorylation. J. Biol. Chem. 277, 25370–25376.
[12] Wood, Z.A., Schröder, E., Harris, J.R. and Poole, L.B. (2003) Structure, mechanism and regulation of peroxiredoxin. Trends Biochem. Sci. 28, 32–40.
[13] Dietz, K.J., Jacob, S., Oelze, M.L., Laxa, M., Tognetti, V., de Miranda, S.M., Baier, M. and Finkemeier, I. (2006) The function of peroxiredoxins in plant organelle, redox metabolism. J. Exp. Bot. 57, 697–709.
[14] Barranco-Medina, S., Kakorin, S., Lázaro, J.J. and Dietz, K.J. (2008) Thermodynamics of the dimer–decamer transition of reduced human and plant 2-Cys peroxiredoxin. Biochemistry 47, 7196–7204.
[15] Bernier-Villamor, L., Navarro, E., Sevilla, F. and Lázaro, J.J. (2004) Cloning and characterization of a 2-Cys peroxiredoxin from *Pisum sativum*. J. Exp. Bot. 55, 2191–2199.
[16] König, J., Baier, M., Horling, F., Kahmann, U., Harris, G., Schürmann, P. and Dietz, K.J. (2002) The plant-specific function of 2-Cys peroxiredoxin-mediated detoxification of peroxides in the redox-hierarchy of photosynthetic electron flux. Proc. Natl. Acad. Sci. USA 99, 5738–5743.
[17] König, J., Lotte, K., Plessow, R., Brockhinke, A., Baier, M. and Dietz, K.J. (2003) Reaction mechanism of plant 2-Cys peroxiredoxin. Role of the C terminus and the quaternary structure. J. Biol. Chem. 278, 24409–24420.
[18] Meissner, U., Schröder, E., Schefler, D., Martin, A.G. and Harris, J.R. (2007) Formation, TEM study and 3D reconstruction of the human erythrocyte peroxiredoxin-2 dodecahedral higher-order assembly. Micron 38, 29–39.
[19] Cao, Z., Roszak, A.W., Gourlay, L.J., Lindsay, J.G. and Isaacs, N.W. (2005) Bovine mitochondrial peroxiredoxin III forms a two-rings catenane. Structure 13, 1661–1664.
[20] Cao, Z., Bhella, D. and Lindsay, J.G. (2007) Reconstitution of the mitochondrial Prx3 antioxidant defence pathway: general properties and factors affecting Prx3 activity and oligomeric state. J. Mol. Biol. 372, 1022–1033.
[21] Jang, H.H., Lee, K.O., Chi, Y.H., Jung, B.G., Park, S.K., Park, J.H., Lee, J.R., Lee, S.S., Moon, J.C., Yun, J.W., Choi, Y.O., Kim, W.Y., Kang, J.S., Cheong, G.W., Yun, D.J., Rhee, S.G., Cho, M.J. and Lee, S.Y. (2004) Two enzymes in one: two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. Cell 117, 625–635.
[22] Lim, J.C., Choi, H.I., Park, Y.S., Nam, H.W., Woo, H.A., Kwon, K.S., Kim, Y.S., Rhee, S.G., Kim, K. and Chae, H.Z. (2008) Irreversible oxidation of the active-site cysteine of peroxiredoxin to cysteine sulfonic acid for enhanced molecular chaperone activity. J. Biol. Chem. 283, 28873–28880.
[23] Koo, H.K., Lee, S., Jeong, S.Y., Kim, E.T., Kim, H.J., Kim, K., Song, K. and Chae, H.Z. (2002) Regulation of thioredoxin peroxidase activity by C-terminal truncation. Arch. Biochem. Biophys. 397, 312–318.
[24] Jang, H.H., Kim, S.Y., Park, S.K., Jeon, H.S., Lee, Y.M., Jung, J.H., Lee, S.Y., Chae, H.B., Jung, Y.J., Lee, K.O., Lim, C.O., Chung, W.S., Bahk, J.D., Jun, D.J., Cho, M.J. and Lee, S.Y. (2006) Phosphorylation and concomitant structural changes in human 2-Cys peroxiredoxin isotype I differentially regulate its peroxidase and molecular chaperone functions. FEBS Lett. 580, 351–355.
[25] Kitano, K., Niimura, Y., Nishiyama, Y. and Miki, K. (1999) Stimulation of peroxidase activity by decamerization related to ionic strength: AhpC protein from *Amphibacillus xylanus*. J. Biochem. 126, 313–319.
[26] Alphey, M.S., Bond, C.S., Tetaud, E., Fairlamb, A.H. and Hunter, W.N. (2000) The structure of reduced tryparedoxin peroxidase reveals a decamer and insight into reactivity of 2Cys-peroxiredoxins. J. Mol. Biol. 21, 903–916.
[27] Hirotsu, S., Abe, Y., Okada, K., Nagahara, N., Hori, H., Nishino, T. and Hakoshima, T. (1999) Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23 kDa/proliferation-associated gene product. Proc. Natl. Acad. Sci. USA 96, 12333–12338.
[28] Chauhan, R. and Mande, S.C. (2001) Characterization of the *Mycobacterium tuberculosis* H37Rv alkyl hydroperoxidase AhpC points to the importance of ionic interactions in oligomerization and activity. Biochem. J. 354, 209–215.
[29] Guimaraes, B.G., Souchon, H., Honore, N., Saint-Joanis, B., Brosch, R., Shepard, W., Cole, S.T. and Alzari, P.M. (2005) Structure and mechanism of the alkyl hydroperoxidase AhpC, a key element of the *Mycobacterium tuberculosis* defence system against oxidative stress. J. Biol. Chem. 280, 25735–25742.
[30] Papinutto, E., Windle, H.J., Cendron, L., Battistutta, R., Kelleher, D. and Zanotti, G. (2005) Crystal structure of alkyl hydroperoxide-reductase (AhpC) from *Helicobacter pylori*. Biochim. Biophys. Acta 1753, 240–246.
[31] Chuang, M.H., Wu, M.S., Lo, W.L., Lin, J.T. and Wong, C.H. (2006) The antioxidant protein alkylhydroperoxide reductase of *Helicobacter pylori* switches from a peroxide reductase to a molecular chaperone function. Proc. Natl. Acad. Sci. USA 103, 2552–2557.
[32] Stork, T., Laxa, M., Dietz, M.S. and Dietz, K.J. (2009) Functional characterization of the peroxiredoxin gene family members of *Synechococcus elongatus* PCC 7942. Arch. Microbiol. 191, 141–151.
[33] Plishker, G.A., Chevalier, D., Seinsoth, L. and Moore, R.B. (1992) Calcium-activated potassium transport and high molecular weight forms of calpromotin. J. Biol. Chem. 267, 21839–21843.
[34] Moore, R.B. and Shriver, S.K. (1997) Protein 7.2b of human erythrocyte membranes binds to calpromotin. Biochem. Biophys. Res. Commun. 232, 294–297.
[35] Kirstensen, P., Rasmussen, D.E. and Kristensen, B.I. (1999) Properties of thiol-specific antioxidant protein or calpromotin in solution. Biochem. Biophys. Res. Commun. 262, 127–131.
[36] Moon, J.C., Han, Y.S., Kim, W.Y., Jung, B.G., Jang, H.H., Lee, J.R., Kim, S.Y., Lee, Y.M., Jeon, M.G., Kim, C.W., Cho, M.J. and Kilner, P.J. (2005) Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa Cell resistance to H_2O_2-induced cell death. J. Biol. Chem. 280, 28775–28784.
[37] Lee, W., Choi, K.S., Riddell, J., Ip, C., Ghosh, D., Park, J.H. and Park, Y.M. (2007) Human peroxiredoxin 1 and 2 are not duplicate proteins: the unique presence of CYS83 in Prx1 underscores the structural and functional differences between Prx1 and Prx2. J. Biol. Chem. 282, 22011–22022.
[38] Matsamura, T., Okamoto, K., Iwahara, S., Hori, H., Takahashi, Y., Nishino, T. and Abe, Y. (2008) Dimer–oligomer interconversion of wild-type and mutant rat 2-Cys peroxiredoxin: disulfide formation at dimer–dimer interfaces is not essential for decamerization. J. Biol. Chem. 283, 284–293.
[39] Akhtar, M.W., Srinivas, V., Raman, B., Ramakrishna, T., Inobe, T., Maki, K., Arai, M., Kuwajima, K. and Rao, Ch.M. (2004) Oligomeric Hsp33 with enhanced chaperone activity: gel filtration, cross-linking, and small angle X-ray scattering (SAXS) analysis. J. Biol. Chem. 279, 55760–55769.
[40] Pena-Ahumada, A., Kahmann, U., Dietz, K.J. and Baier, M. (2006) Regulation of peroxiredoxin expression versus expression of Halliwell–Asada-Cycle enzymes during early seedling development of *Arabidopsis thaliana*. Photosynth. Res. 89, 99–112.
[41] Baier, M. and Dietz, K.J. (1996) Primary structure and expression of plant homologues of animal and fungal thioredoxin-dependent peroxide reductases and bacterial alkyl hydroperoxide reductases. Plant Mol. Biol. 31, 553–564.
[42] Baier, M. and Dietz, K.J. (1999) Protective function of chloroplast 2-cysteine peroxiredoxin in photosynthesis. Evidence from transgenic Arabidopsis. Plant Physiol. 119, 1407–1414.
[43] Caporaletti, D., D'Alessio, A.C., Rodriguez-Suarez, R.J., Senn, A.M., Duek, P.D. and Wolosiuk, R.A. (2007) Non-reductive modulation of chloroplast fructose-1,6-bisphosphatase by 2-Cys peroxiredoxin. Biochem. Biophys. Res. Commun. 355, 722–727.
[44] Aran, M., Caporaletti, D., Senn, A.M., Tellez de Iñon, M.T., Girotti, M.R., Llera, A.S. and Wolosiuk, R.A. (2007) ATP-dependent modulation and autophosphorylation of rapeseed 2-Cys peroxiredoxin. FEBS Lett. 275, 1450–1463.
[45] Evrard, C., Capron, A., Marchand, C., Clippe, A., Wattiez, R., Soumillion, P., Knoops, B. and Declerq, J.P. (2004) Crystal structure of a dimeric oxidized form of human peroxiredoxin 5. J. Mol. Biol. 337, 1079–1090.
[46] Smeets, A., Machand, C., Linard, D., Knoops, B. and Declercq, J.P. (2008) The crystal structures of oxidized forms of human peroxiredoxin 5 with an intramolecular disulfide bond confirms the proposed enzymatic mechanisms for atypical 2-Cys peroixiredoxin. Arch. Biochem. Biophys. 447, 98–104.
[47] Barranco-Medina, S., Krell, T., Bernier-Villamor, L., Sevilla, F., Lázaro, J.J. and Dietz, K.J. (2008) Hexameric oligomerization of mitochondrial peroxiredoxin PrxIIF and formation of an ultrahigh affinity complex with its electron donor thioredoxin Trx-o. J. Exp. Bot. 59, 3259–3269.
[48] Barranco-Medina, S., Lopez-Jaramillo, F.J., Bernier-Villamor, L., Sevilla, F. and Lázaro, J.J. (2006) Cloning, overexpression, purification and preliminary crystallographic studies of a mitochondrial type II peroxiredoxin from *Pisum sativum*. Acta Crystallogr. Sect. F 62, 695–698.
[49] Kim, S.J., Woo, J.R., Hwang, Y.S., Jeong, D.G., Shin, D.H., Kim, K. and Ryu, S.E. (2003) The tetrameric structure of *Haemophilus influenza* hybrid Prx5 reveals interaction between donor and acceptor proteins. J. Biol. Chem. 278, 10790–10798.
[50] Baker, L.M. and Poole, L.B. (2003) Catalytic mechanism of thiol peroxidase from *Escherichia coli*, sulfenic acid formation and overoxidation of essential CYS61. J. Biol. Chem. 278, 9203–9211.
[51] Choi, J., Choi, S., Choi, J., Cha, M.K., Kim, I.H. and Shim, W. (2003) Crystal structure of *Escherichia coli* thiol peroxidase in the oxidized state: insights into intramolecular disulfide formation and substrate binding in atypical 2-Cys peroxiredoxins. J. Biol. Chem. 278, 49478–49486.
[52] Lu, J., Yang, F., Li, Y., Zang, X., Xia, B. and Jin, C. (2008) Reversible conformational switch revealed by the redox structures of *Bacillus subtilis* thiol peroxidase. Biochem. Biophys. Res. Commun. 373, 414–418.
[53] Choi, H.J., Kang, S.W., Yang, C.H., Rhee, S.G. and Ruy, S.E. (1998) Crystal structure of a novel human peroxidase enzyme at 2.0 Å resolution. Nat. Struct. Biol. 5, 400–406.
[54] Wu, Y.Z., Manevich, Y., Baldwin, J.L., Dodia, C., Yu, K., Feinstein, S.I. and Fisher, A.B. (2006) Interaction of surfactant protein A with peroxiredoxin 6 regulates phospholipase A2 activity. J. Biol. Chem. 281, 5715–7525.

[55] Deponte, M. and Becker, K. (2004) Biochemical characterization of *Toxoplasma fondii* 1-Cys peroxiredoxin 2 with mechanistic similarities to typical 2-Cys Prx. Mol. Biochem. Paras. 140, 87–96.
[56] Jeon, S.J. and Ishikawa, K. (2003) Characterization of novel hexadecameric thioredoxin peroxidase from *Aeropyrum pernix* K1. J. Biol. Chem. 278, 24174–24180.
[57] Mizohata, E., Sakai, H., Fusatomi, E., Terada, T., Murayama, K., Shirouzu, M. and Yokoyama, S. (2005) Crystal structure of an archaeal peroxiredoxin from the aerobic hyperthermophilic crenarchaeon *Aeropyrum pernix* K1. J. Mol. Biol. 354, 317–329.
[58] Smeets, A., Loumaye, E., Clippe, A., Rees, J.F., Knoops, B. and Declercq, J.P. (2008) The crystal structure of the C45S mutant of annelid *Arenicola marina* peroxiredoxin 6 supports its assignment to the mechanistically 2-Cys subfamily without any formation of toroid-shaped decamers. Protein Sci. 4, 700–710.
[59] Bystrova, M.F., Budanova, E.N., Novoselov, V.I. and Fesenko, E.E. (2007) Study of the quarternary structure of rat 1-Cys peroxiredoxin. Biofzika 52, 436–442.
[60] Li, S., Peterson, N.A., Kim, M.Y., Kim, C.Y., Hung, L.W., Yu, M., Lekin, T., Segelke, B.W., Lott, J.S. and Baker, E.N. (2005) Crystal structure of AhpE from *Mycobacterium tuberculosis*, a 1-Cys peroxiredoxin. J. Mol. Biol. 346, 1035–1046.
[61] Noguera-Mazon, V., Lemoine, J., Walker, O., Rouhier, N., Salvador, A., Jacquot, J.P., Lancelin, J.M. and Krimm, I. (2006) Glutathionylation induces the dissociation of 1-Cys-peroxiredoxin non-covalent homodimer. J. Biol. Chem. 281, 31736–31742.
[62] Ralat, L.A., Misquitta, S.A., Manevich, Y., Fisher, A.B. and Colman, R.F. (2008) Characterization of the complex of glutathione *S*-transferase pi and 1-cysteine peroxiredoxin. Arch. Biochem. Biophys. 474, 109–118.
[63] Monteiro, G., Horta, B.B., Pimenta, D.C., Augusto, O. and Netto, L.E. (2007) Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. Proc. Natl. Acad. Sci. USA 104, 4886–4891.
[64] Klem, M.T., Young, M. and Douglas, T. (2005) Biomimetic magnetic nanoparticles. Mater. Today, 28–37.