ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 3, 2011

DOI: 10.1089/ars.2010.3397

FORUM REVIEW ARTICLE

A Comparison of Thiol Peroxidase Mechanisms

Leopold Flohé,[1] Stefano Toppo,[2] Giorgio Cozza,[2] and Fulvio Ursini[2]

Abstract

Thiol peroxidases comprise glutathione peroxidases (GPx) and peroxiredoxins (Prx). The enzymes of both families reduce hydroperoxides with thiols by enzyme-substitution mechanisms. H_2O_2 and organic hydroperoxides are reduced by all thiol peroxidases, most efficiently by SecGPxs, whereas fast peroxynitrite reduction is more common in Prxs. Reduction of lipid hydroperoxides is the domain of monomeric GPx4-type enzymes and of some Prxs. The catalysis starts with oxidation of an active-site selenocysteine (U_P) or cysteine (C_P). Activation of Cys (Sec) for hydroperoxide reduction in the GPx family is achieved by a typical tetrad composed of Cys (Sec), Asn, Gln, and Trp, whereas a triad of Cys Thr (or Ser) and Arg is the signature of Prx. In many of the CysGPxs and Prxs, a second Cys (C_R) is required. In these 2-CysGPxs and 2-CysPrxs, the C_P oxidized to a sulfenic acid forms an intra- or intermolecular disulfide (typical 2-CysPrx) with C_R, before a stepwise regeneration of ground-state enzyme by redoxin-type proteins can proceed. In SecGPxs and sporadically in Prxs, GSH is used as the reductant. Diversity combined with structural variability predestines thiol peroxidases for redox regulation via ROOH sensing and direct or indirect transduction of oxidant signals to specific protein targets. *Antioxid. Redox Signal.* 15, 763–780.

Introduction

PEROXIDASES THAT REDUCE HYDROPEROXIDES at the expense of thiols belong to two huge protein families, which are phylogenetically poorly, if at all, interrelated. The first one came on stage in 1957 with the discovery of glutathione peroxidase (GPx) by Gordon C. Mills (80), an enzyme that catalyzes GSH oxidation by H_2O_2 and a variety of other hydroperoxides and became the first selenoprotein to be identified in mammals (31, 37, 97). The specificity of this prototype gave its name to the entire family, the "glutathione peroxidases," although most of its nonmammalian relatives use thioredoxin or related redoxins as reductants (77).

The emergence of the other family, the peroxiredoxins (Prx), is more diffuse. This kind of protein was first described in 1968 by J. Robin Harris (52) as ring-shaped protein complex of unknown function and was called "torin"; later as a variety of "factors" implicated mostly in immunology [reviewed in (35)], as "thiol-specific antioxidant protein" of yeast (63), which for the first time was correctly annotated as thioredoxin peroxidase by Sue Goo Rhee and his group in 1994 (13), as a component of the alkylhydroperoxide reductase system in enterobacteria (112), and, in search of a nonexistent trypanothione peroxidase, as "tryparedoxin peroxidase" in kinetoplasts (89). Also to Sue Goo Rhee we owe the insight that these seemingly heterogeneous proteins build a protein family that is defined by sequence homology and phylogenetic relatedness and so are called peroxiredoxins (14).

The common denominator of the two families of enzymes is their ability to reduce a broad spectrum of hydroperoxides, including peroxynitrite, with the concomitant oxidation of two thiol groups to a disulfide. The two thiols may be presented by two molecules, as in the typical GPx reaction (Eq. 1), or by a single molecule, as in a thioredoxin peroxidase reaction (Eq. 2).

$$ROOH + 2\ GSH \rightarrow GSSG + ROH + H_2O \quad (1)$$

$$ROOH + Trx(SH)_2 \rightarrow TrxS_2 + ROH + H_2O \quad (2)$$

In common also is their kinetic mechanism, which is an "enzyme-substitution mechanism," as revealed by ping-pong kinetics. This implies that the catalytic mechanisms do not involve any central complexes of the enzymes with all the substrates bound simultaneously. Instead, the catalytic cycles are composed of sequences of bimolecular reactions between the enzymes and their substrates. None of the families requires any cofactor or prosthetic group, such as heme or flavin, for peroxidase activity; they act as naked proteins in which cysteines or selenocysteines undergo redox shuttling (14, 115, 117). Differences between the families exist in the way they achieve specificity for the reducing substrate, although in this respect, variations within the families appear

[1]Otto-von-Guericke-Universität and MOLISA GmbH, Magdeburg, Germany.
[2]Department of Biological Chemistry, University of Padova, Padova, Italy.

as pronounced as do those between them. Clearly distinct, however, is the mode of activation of the active-site chalcogen for the reaction with ROOH.

The enzymology of GPx- and Prx-type peroxidases has amply been reviewed in recent articles (12, 24, 33, 34, 54, 56, 57, 92, 114-117). The present Forum review therefore focuses on analogies, similarities, and differences between the enzyme families.

What Makes a Protein a Peroxidase?

For more than half a century, the only known peroxidases were heme-containing proteins that used the complexed transition metal iron for attacking H_2O_2 (36), a mechanism that chemically appeared plausible. With the prototype GPx1 (80), the GPx family was the first to break this rule, and, with a delay of four decades, the peroxiredoxins followed with the discovery of the thioredoxin peroxidase activity of the "thiol-specific antioxidant protein" of yeast (13, 88). Why these proteins are efficient peroxidases is by no means a trivial question. When selenium was discovered in GPx1, the problem seemed to be solved by referring to the magic catalytic power of this element (31), and this view was corroborated by a substantial decrease of specific activity when the selenocysteine residue (Sec) in GPxs was changed to cysteine by site-directed mutagenesis (76, 96).

In the meantime, however, GPxs that naturally have the active-site Sec replaced by Cys were discovered and proved to be quite efficient peroxidases (115). With rate constants k_{+1} for the reaction with hydroperoxides of about 10^6 $M^{-1}s^{-1}$, they approach the peroxidatic efficiencies of their Sec-containing relatives (77). Similarly, Prxs, which have long been considered to be comparatively sluggish peroxidases (56), are increasingly reported to reach peroxidatic efficiencies almost overlapping with those of SecGPxs (117), and only exceptionally do they contain Sec (107). Moreover, Sec-containing proteins are found in almost all living domains (with the exception of higher plants and yeasts); only a minority of those are peroxidases (46), and surface-exposed cysteines are present in many proteins without lending them any significant peroxidase activity. In short, (a) selenium versus sulfur makes a quantitative, but not a qualitative difference in catalytic efficiency; and (b) it must be the particular environment of the redox-active residues Cys or Sec that facilitates their reaction with an ROOH.

In analogy to an early hypothesis on the GPx reaction (28), the first step of the Prx catalysis is seen in the oxidation of a particular Cys residue, which here is often embedded in a VCP motif and considered to be dissociated, to a sulfenic acid (Eq. 3).

$$\mathrm{Prx-S^- + ROOH \rightarrow Prx-SO^- + ROH} \qquad (3)$$

This residue of primary hydroperoxide attack is called the peroxidatic cysteine (C_P).

In a series of elegant investigations by Leslie B. Poole (6, 26, 92–94), the chemical nature of the oxidized C_P could indeed be demonstrated to be a sulfenic acid by mass spectrometry, and this labile oxidation state of C_P also survived the crystallography of human ORF06/Prx6 (15). An analogous intermediate could be identified in the yeast Orp1 protein, which is a CysGPx, and is generally assumed to be the first intermediate of the catalytic cycle of CysGPxs. In SecGPxs, hardly any alternative exists to the assumption of a homologous oxidation of the Sec selenolate to a selenenic acid (Eq. 4).

$$\mathrm{GPx-Se^- + ROOH \rightarrow GPx-SeO^- + ROH} \qquad (4)$$

Despite major efforts, however, this hypothetical catalytic intermediate could not be verified. Instead, whenever a SecGPx was exposed to a just stoichiometric amount of H_2O_2, mass spectrometry revealed that the oxidized enzyme is smaller than the reduced one by two mass units, indicating that the presumed primary oxidation product has lost a water molecule (78). The selenenic acid form of these enzymes thus resembles a transition state that is instantly transformed into a more-stable derivative of the same oxidation state, the structure of which still remains elusive (see later).

To make the partial reactions 3 and 4 happen with a satisfying bimolecular rate constant k_{+1}, the following prerequisites are mandatory: (a) the active site Cys or Sec, respectively, has to be dissociated, because thiols or selenols will not readily react with a hydroperoxide; (b) the peroxyl bond has to be polarized to facilitate a nucleophilic attack of the thiolate (selenolate) on the more-positive oxygen; and (c) a mobile proton has to create an ROH (or H_2O, if the substrate was H_2O_2) as a strong leaving group from the RO^- of the cleaved peroxyl bond. How these requirements are met by the enzymes is not entirely clear. The architecture of their active sites, however, allows formulating hypotheses that in part have been corroborated experimentally.

The structural signature of all peroxiredoxins is a triad built of the C_P, with its sulphur being coordinated to the guanidino group of an arginine and the OH of a threonine (or sporadically a serine) (40, 51, 62). The C_P and the Thr(Ser) are part of the motif PxxxT(S)xxC, which is strictly conserved in the entire family, whereas the equally conserved Arg is remotely localized in sequence (Fig. 1). That the conserved Cys near the N-terminus is indeed the peroxidatic one is evident from complete abrogation of activity by its exchange against Ser or other residues, as shown in many species (92). The functional essentiality of the highly conserved Thr was first demonstrated

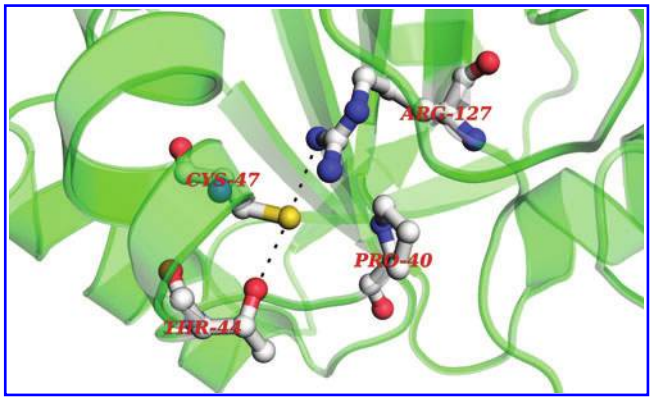

FIG. 1. Catalytic triad of a Prx built up from Cys, Thr, and Arg. The C_P sulfur in all reduced Prxs is coordinated (*dotted lines*) to an Arg and a Thr (or Ser). A strictly conserved Pro stabilizes the triad. The example shown is human Prx5 (pdb:1HD2), as established by Declercq *et al*. (20). Figure obtained by using Pymol (http://www.pymol.org/). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

in the tryparedoxin peroxidase of *Leishmania donovani*. A valine in this position practically abrogated activity, whereas a serine, which is the only natural alternative found in a few species, even enhanced it, which underscores the importance of a sulfur-coordinated OH group (40). The conserved Arg in the more C-terminal part of the sequence was found to be equally essential: Replacement by acid residues in *Crithidia fasciculata* (82) and *Leishmania donovani* (38) abolished activity, whereas a less dramatic exchange against Gln in barley Prx substantially reduced activity (67). The conserved Pro upstream of the triad Thr is considered to stabilize the position of the active-site structure by sustaining a hydrogen bridge between its prolyl-amide nitrogen and the C_P sulphur (62). Collectively, these findings support the view that the threonine (serine) OH is hydrogen-bonded to the C_P sulfur, thereby facilitating dissociation of its SH group, and that the resulting thiolate is stabilized by a bi-dentate salt bridge with the conserved Arg (92).

The conserved microarchitecture of the C_P environment thus enforces a proton dislocation from the C_P thiol and thereby allows the reaction with a hydroperoxide. For several Prxs, pK_a values near 6 were indeed determined, provided that the C_P environment be correctly folded (87). However, fulfilling this basic requirement of the catalysis is by no means sufficient to explain the efficiency of Prxs. Known bimolecular rate constants for the reaction of low-molecular-weight thiol compounds with ROOH, even when extrapolated to full dissociation, do not exceed 30 $M^{-1}s^{-1}$ (124), whereas the corresponding k_{+1} values for Prxs cover a range from 2×10^3 to 2×10^7 $M^{-1}s^{-1}$ (117), and in the reaction with peroxynitrite, they even reach 7×10^7 $M^{-1}s^{-1}$ (25). The substantial gap in reactivity has to be bridged in the enzymes by polarizing the substrate's peroxyl-bond and providing a proton to the leaving group. Whether the triad components or additional, less conserved residues are hereby involved remains a matter of speculation.

In the GPx family, the analogous C_P activation is achieved by similar principles but totally different amino acids and, notably, without any net positive charge near its sulfur or selenium. Here the C_P sulfur or U_P selenium, respectively, is located in a NVAxxC(U)G motif near the N-terminus. In the 3-D structure, the C_P (U_P) is surrounded by the last Gln of a central FPCNQFGxQ motif and by a strictly conserved C-terminal WNF motif (Fig. 2).

The proximity of the selenium in bovine GPx1 to the carboxamide group of the central Gln and the imino group of the C-terminal Trp in the first GPx structure ever established had already suggested a functional relevance of these residues (27). This assumption was first verified by mutagenesis studies with porcine GPx4 (76): In the Sec→Cys mutant, the k_{+1} decreased by a factor of 300, revealing again the superior catalytic efficiency of selenium and corroborating that the Sec or Cys in this position is U_P or C_P, respectively. When the presumably critical residues Gln and Trp were mutated in GPx4Sec→Cys, the k_{+1} values further decreased, and acidic residues instead of Gln and Trp proved to be most detrimental. The decrease in reactivity was largely paralleled by a decrease in the rate of alkylation by iodoacetate of the U_P and C_P, respectively, which also depends on their pK_a. This finding unambiguously demonstrated that the active-site residues Gln and Trp indeed reduce the pK_a of U_P and C_P. Interestingly, however, the rates of inactivation by iodoacetate of both the wild-type enzyme and the Cys mutant were not only highest, but also very similar, underscoring that not only Sec,

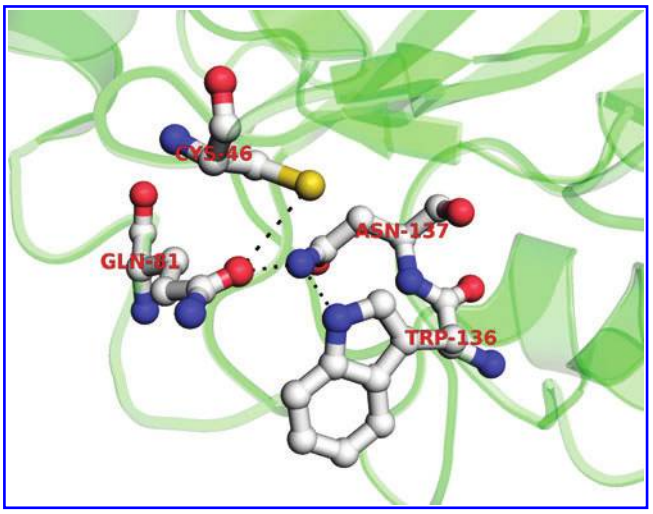

FIG. 2. Catalytic tetrad of a GPx. The residues Cys (or Sec), Gln, Asn, and Trp are highly conserved in the GPx family. Possible polar contacts between the tetrad residues and the C_P (U_P) sulfur (selenium) are highlighted (*dotted lines*). Residue numbering corresponds to human GPx4 pdb: 2OBI (103). Figure was obtained by using Pymol. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

which as free selenocysteine has a pK_a around 5, but also the less-acidic Cys is practically dissociated in the peculiar environment of the enzyme. Similar results were obtained by mutagenesis studies with natural CysGPxs, GPxIII of *Trypanosoma brucei* (104) and Orp1/GPxIII of *Saccharomyces cerevisiae* (73). Based on these studies, the "catalytic triad" composed of Sec(Cys), Gln, and Trp became the typical signature of the entire family. In a GPx of Chinese cabbage (60), however, the Gln could be mutated to Gly without loss of activity. In the *T. brucei* GPxIII, the effects of Trp mutation were not impressive, questioning a pivotal role of the Trp in catalysis (104). Some findings with natural GPxs further challenged the triad dogma: In the poplar GPx, which is an active thioredoxin peroxidase, the triad Gln is exchanged against Glu (65); and in human GPx8, whose function is still elusive, the Gln is replaced by a Ser (114). More recently, a study on GPx of *Drosophila melanogaster* revealed that the Asn residue of the WNF motif, which reaches the C_P sulphur from the core of the protein with its carboxamide group, has a greater impact on C_P dissociation and the k_{+1} value than any of the other triad residues, which led the authors propose to enlarge the GPx triad to a "tetrad" (116) (Fig. 2).

Collectively, the Trp, Gln, and Asn residues of the GPx tetrad contribute to the C_P dissociation, Trp being the least- and Asn the most-important one, and in special rare cases, the tetrad components may substitute for each other in this function. In contrast to Prx catalysis, C_P dissociation in GPx is guaranteed without any strong base. For SecGPxs, a nearby basic residue, such as the Arg in Prxs, is not required, because Sec is dissociated anyway at physiologic pH. But what reduces the C_P pK_a in a CysGPx, which has been calculated (3) to be around 7 in *Dm*GPx (116) or inferred from alkylation studies (73) to approach 5 in Orp1? Evidently it is the density of labile protons provided by the two carboxamide groups, which, depending on the rotamer and tautomer assumed, are

close enough to form hydrogen bridges, even low-barrier hydrogen bonds, or to enable proton shuttling between each other and toward the C_P sulphur. A recent state-of-the-art quantum chemistry computational approach corroborates that the C_P/U_P proton is indeed completely dislocated and may shuttle between the imino and amido functions of the GPx tetrad, irrespective of being derived from Sec or Cys (Orian and Ursini, unpublished data).

The present state of related knowledge and discussions is best summarized as follows (115, 116):

1. The carboxamide of the core-hidden Asn attracts the thiol proton of C_P. In the resulting low-barrier hydrogen bond, the proton may shuttle between the amide of Asn and the sulphur of C_P, a situation that is practically equivalent to a salt bridge between the positively charged, since protonated, amide and the negative thiolate.
2. A similar role may be assumed for the carboxamide of the Gln. Because this residue is much more surface exposed, it is likely more relevant to the polarization of the substrate's peroxyl-bond ($ROOH \rightarrow RO^{\delta-}\text{-}\ O^{\delta+}H$) by analogous proton shuttling.
3. The negative C_P sulfur attacks the more positive oxygen of the polarized peroxyl-bond.
4. The proton required to cleave the peroxyl-bond by creating a strong leaving group ($RO^- \rightarrow ROH$) is most likely delivered by the Gln carboxamide.
5. The primary role of the Trp is seen as keeping the C_P sulfur in place *via* its imino nitrogen. Thus, despite the absence of any residue in the GPx tetrad that bears a net positive charge at physiologic pH, the C_P oxidation is in essence an acid/base catalysis, as is also hypothesized for Prx.

Comments on Hydroperoxide Specificities

GPxs as well as Prxs are known to be broad-spectrum peroxidases acting on H_2O_2, primary, secondary, and tertiary organic hydroperoxides, and peroxynitrite. Typically, the reaction rates decline from H_2O_2 over primary and secondary toward tertiary peroxides in GPx (50, 115) and Prx (90, 102, 117), whereas those for peroxynitrite appear to be particularly high in some of the Prxs (25, 117). Reports on lack of activity of these enzymes with H_2O_2 should be considered with caution, because it may result from fast inactivation due to C_P overoxidation (see later). In the GPx family, efficient reduction of hydroperoxides of complex lipids that tend to form micelles or are integrated into biomembranes was first reported for pig GPx4, the "phospholipid hydroperoxide glutathione peroxidase" (119), and later for similar monomeric CysGPxs (115), whereas it is low or absent in tetrameric GPxs (111, 115). In the Prx family, activity on phospholipid hydroperoxides was first observed with tryparedoxin peroxidase (89) and later with many other, although not all, Prxs without any obvious prevalence in the subfamilies (117).

A structural basis for the variable hydroperoxide specificity is not unambiguously detectable. For mammalian GPx4, the monomeric character that implies a better accessibility of the enzyme active-site Se has for long been discussed to allow a better interaction of the enzyme with lipid surfaces (120), and an extended positively charged surface area near the reaction center has been suggested to attract the negative charges of phospholipids (115). Otherwise, the rates of hydroperoxide reduction appear to reflect little else than the sterical accessibility of the peroxyl group. None of the thiol peroxidase structures discloses features that could be addressed as specific hydroperoxide-binding pockets. The sulfur or selenium atoms to be oxidized are located in flat wells (GPx) or troughs (Prx), which give access to the hydroperoxyl of most diverse structures such as fatty acids, steroids, nucleic acids, or lipid membranes, as compiled for GPx in 1989 (29) and more recently for Prxs (92). This broad specificity spectrum of the thiol peroxidases is incompatible with the assumption of any typical complex to be formed between the enzymes and their hydroperoxide substrates. The kinetics of these enzymes do not suggest any enzyme hydroperoxide complex either. Maximal velocities and K_M values are infinite for all SecGPxs so far investigated and for many of the CysGPxs and Prxs, and finite $K_{M\ ROOH}$ values, be they apparent or real, can by no means be interpreted as measure for "hydroperoxide affinity," as in countless publications (this is unjustified in the ping-pong mechanism anyway and certainly wrong in case of the thiol peroxidases). If "hydroperoxide saturation" is observed in a steady-state kinetics analysis of a thiol peroxidase, it does not mean that its active site is fully occupied by the hydroperoxide substrate but that, under the prevailing conditions, no reduced enzyme is left to be oxidized. In other words, the rate constants k_{+1} for the representative Eqs. 3 and 4 are real bimolecular rate constants for the reaction of C_P (U_P) and ROOH, and their values are in essence determined by the reactivity of the U_P or C_P, respectively, sterical accessibility and reactivity of the substrate's peroxyl group, and the resulting collision efficiency.

Tricks to Save the Oxidation State of Labile Catalytic Intermediates

As outlined earlier, the products of the first step of GPx and Prx catalysis, apart from water or an alcohol, are oxidatively modified enzymes, having exposed most reactive selenenic or sulfenic acid functions. These catalytic intermediates have to be very reactive to be prepared for fast regeneration of the ground-state enzymes. However, this catalytic prerequisite also implies that the intermediates are unstable, because they might react with quite a lot of abundant molecules, if they do not meet their appropriate target substrates in time. The most common side reaction of the intermediates is overoxidation of the selenenic or sulfenic acids to seleninic and sulfinic acids, respectively. Surprisingly, however, the inherent chemical instability of the catalytic intermediates does not result in any unusually fast inactivation or denaturation of Prxs or GPxs. Evidently, nature has taken measures to prevent detrimental side reactions.

The enigmatic nature of oxidized SecGPx

The most-unstable intermediate implicated in the catalysis of thiol peroxidases is the selenenic acid form of a SecGPx (see Eq. 4). A reasonably stable organic selenenic acid has so far been synthesized only once, and, in this case, the selenenic acid function was shielded from potential reaction partners in a cage-like molecule (48), whereas in the oxidized SecGPxs, it has to be surface exposed to fulfil its catalytic role and, accordingly, is prone to become overoxidized to a seleninic acid. In the x-ray structure of GPx1 the active site Se had indeed two oxygen atoms attached (27). Also, sluggish forms of GPx1,

which are observed on storage of the enzyme under oxidative conditions and require long-term preincubation with thiols to become fully active again (72, 109), have been interpreted to have the Se overoxidized to a seleninic acid (29). How, then, are these suicidal reactions prevented during the normal catalytic cycle?

As mentioned, the first *detectable* oxidation products of SecGPx4 (78) and SecGPx1 (Ursini and Mauri, unpublished data) were not the presumed selenenic acid forms, but corresponded to this theoretically postulated intermediate after elimination of water. H_2O elimination from a selenenic acid residue may be envisaged to result from a reaction with selenols or thiols. However, formation of a diselenide bridge between two GPx molecules is incompatible with the observed molecular weight of the oxidized SecGPxs, nor is this possibility suggested by molecular modeling or docking calculations (78). An intramolecular selenyl-sulfide bond would be compatible with the observed molecular weight, but neither the structures of GPx4 (103) nor those of GPx1 (27) and GPx3 (95) reveal any thiols that are in reach of the selenenic acid function. A third explanation of the phenomenon would be a reaction of the selenenic acid with an amine or amide in the catalytic center. That this idea is more than an undue speculation is supported by the reaction cycle of the GPx mimic ebselen [2-phenyl-1,2-benzisoselenazol-3(2H)-one]. This compound is formed by oxidation of an aromatic selenol with a carboxamide function in the ortho position, whereby a transiently formed selenenic acid reacts with the carboxamide nitrogen and undergoes ring closure with elimination of H_2O. In the resulting selenazolinone, the oxidation state of the selenenic acid is conserved in the form of a selenyl-amide bond, but in contrast to a selenenic acid, the selenyl-amide is reasonably stable (Eq. 5)

$$R-SeOH+R'-NH_2 \rightarrow R-Se-NH-R'+H_2O \quad (5)$$

Nevertheless, its Se-N bond is easily cleaved reductively by GSH to regenerate the selenol (101) (Eqs. 6 and 7).

$$R-Se-NH-R'+GSH \rightarrow R-SeSG+H_2N-R' \quad (6)$$

$$R-SeSG+GSH \rightarrow R-SeH+GSSG \quad (7)$$

With regard to the amply available amide groups near the active-site Se in GPxs, a homologous protection of the selenenic acid level from overoxidation during regular catalysis may reasonably be assumed. So far, however, the amide group involved resisted identification.

Disulfide formation in CysGPx

The first oxidation product of a CysGPx, the sulfenic acid formed in analogy to Eq. 3, is more stable than the selenenic acid of its seleno homologue, and it may be questioned whether a protection against overoxidation is at all required. Nevertheless, a sulfenyl-amide formation, in analogy to the ebselen cycle, could be envisaged. Such conservation of the redox status of sulfenic acids has indeed been documented for the protein tyrosine phosphatase PTP1B (100, 122), and also here, the regeneration of the thiol from the sulfenyl-amide form by GSH proved to be possible. Whether analogous reactions occur in CysGPxs (or Prxs) remains to be demonstrated.

For the many CysGPxs with redoxin specificity, a different protection mechanism evolved (Fig. 3). As in 2-Cys peroxiredoxins, the C_P sulfenic acid reacts with a second Cys (C_R) to form a disulfide bond (Eq. 8).

$$GPx(C_P-SOH)C_R-SH \rightarrow GPxS_2+H_2O \quad (8)$$

In the reduced as well as in the sulfenic acid form, this second Cys is far away from the C_P, which implies that here disulfide formation is by no means a trivial process. The coreacting cysteine is embedded in an apparently mobile loop that, on major structural changes, including local unwinding of helices, can interact with the primary reaction center. Thereby, the C_P is turned out from the catalytic tetrad, the latter becoming completely disrupted (Fig. 4). How these extensive movements are enforced is unknown. They appear not to depend on the oxidation of C_P, because the "disulfide conformation" has also been seen with NMR analysis with a reduced enzyme, a mutant but fully active tryparedoxin peroxidase, PxII of *Trypanosoma brucei* (79, 84). Evidently, the tetrad structure is less rigid than suggested by crystallographic data and molecular dynamics (5), and both reduced forms, that with an intact tetrad and the one resembling the conformation of the disulfide form, can coexist in solution. That the seemingly unlikely conformational changes actually happen during catalysis has meanwhile been demonstrated for several CysGPx species by mass spectrometry (22, 73, 77, 104) and comparative structural analysis of the reduced and oxidized enzymes (65). Disulfide formation in this redoxin-specific GPx subfamily is assumed primarily to provide an ideal structural basis for the attack by classic disulfide reductants, such as thioredoxin or tryparedoxin (see later). As in 2-Cys-Prxs, the second Cys is indeed required for the

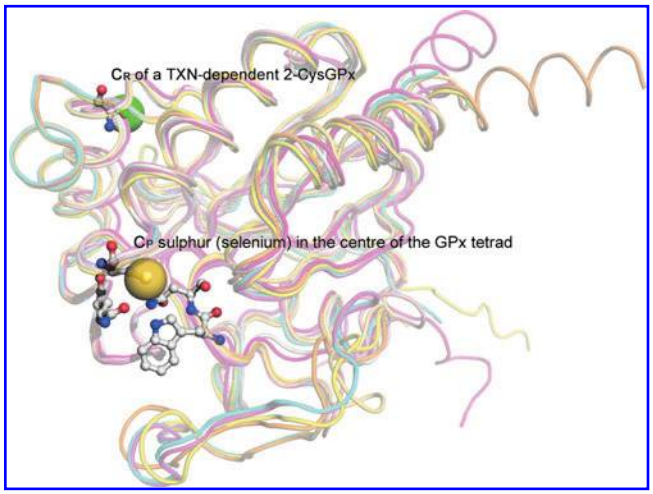

FIG. 3. Overlay of human GPx structures and a 2-CysGPx-type tryparedoxin peroxidase from *Trypanosomama brucei* (*Tb*TXNPx). *Hs*GPx1:pdb 2F8A; *Hs*GPx-2:pdb 2HE3; *Hs*GPx-3: pdb 2R37; *Hs*GPx-4:pdb 2OBI; *Hs*GPx-5: pdb 2I3Y; *Hs*GPx-7:pdb 2P31; *Hs*GPx-8:pdb 3CYN; *Tb*TXNPx: pdb 2VUP (1). The tetrad residues (from 2OBI) and resolving Cys (C_R; from 2VUP) are shown (*ball and sticks*). Figure was obtained by using Pymol. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

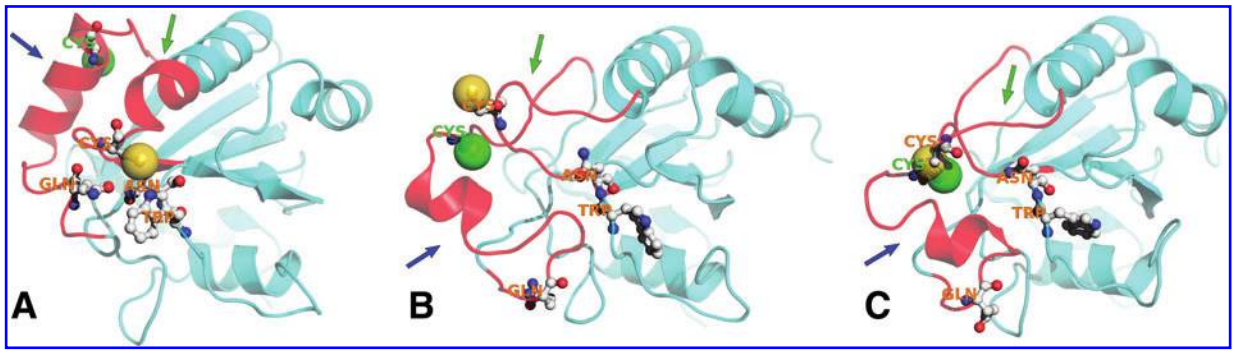

FIG. 4. Structural flexibility of 2-CysGPx. (A) X-ray structure of the reduced form of a tryparedoxin peroxidase of *T. brucei* *Tb*PxII (pdb:2VUP), showing the canonic GPx tetrad (1). **(B)** NMR structure of reduced *Tb*PxIIIC76S (2RM6), which, apart from the C76→S mutation, differs from *Tb*PxII only by a few N-terminal residues. Notice the disruption of the catalytic tetrad (79). **(C)** X-ray structure of oxidized disulfide form of *Tb*PxIII (pdb: 2RM5) closely resembling the conformation shown in B. (Blue arrow) The α-helix carrying the C_R (S in green); (green arrow) the C_P helix (S in yellow). Both helices are widely unfolded in the transition from **A** to **B** and **C** (changing parts highlighted in red). Figure was obtained by using Pymol. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

progression of the catalytic cycle and, accordingly, is called the "resolving" cysteine, C_R.

However, lending redoxin specificity to these GPxs is not the only role of their C_R; the C_R also appears to prevent overoxidation of C_P to a sulfinic acid, which appears to be a common phenomenon in CysGPxs (116) that is substantially enhanced if C_R is mutated into a redox-inactive residue (104).

Disulfide formation in 2-CysPrx

Overoxidation of C_P to sulfinic acid is also a common phenomenon in the Prx family, although substantial differences in susceptibility emerged. As a rule, bacterial Prxs are more resistant than those of higher organisms (126, 127). However, the susceptibility to inactivation by hydroperoxides can markedly differ between orthologous Prxs from one and the same genus or family and also depends on the hydroperoxide substrate in an as-yet poorly understood manner (40). In many eukaryotes, the pronounced susceptibility to inactivation is also counteracted by specific enzymes, the sulfiredoxins, which reduce the sulfinic Prx forms back to active peroxidases (9, 59, 125).

As in CysGPx-type redoxin peroxidases, disulfide formation between C_P and C_R, which initiates the reductive part of the catalytic cycle in 2-CysPrxs (62, 92, 127), also appears to be the preferred mechanism to prevent overoxidation of the sulfenic forms. In 1-CysPrxs, being devoid of a C_R, protection of the sulfenic acid form by intramolecular disulfide formation is impossible. Instead, a mixed disulfide with, *e.g.*, GSH may be envisaged, as is proposed for human Prx VI (105). The reaction scheme of atypical 2-CysPrxs is essentially identical to that of CysGPx-type redoxin peroxidases (compare Eqs. 8 and 9).

$$(\text{atypical } 2-\text{Cys})\text{Prx}(C_P-\text{SOH})C_R-\text{SH} \rightarrow \text{Prx}S_2 + H_2O \quad (9)$$

In the typical 2-CysPrxs, the C_R is out of reach for a reaction with C_P and, instead, two disulfide bridges are formed between the C_Ps and C_Rs within a dimer of head-to-tail–oriented subunits (Eq. 10).

$$(\text{typical } 2-\text{Cys})[\text{Prx}(C_P-\text{SOH})C_R-\text{SH}]_2 \rightarrow (\text{Prx}S_2)_2 + 2\ H_2O \quad (10)$$

As in the CysGPx-type redoxin peroxidases, the C_R/C_P distances in reduced Prxs are very large (Fig. 5). Also, in atypical 2-CysPrxs, the localiation of C_R differs, which has prompted a Prx classification into C, L, and R subfamilies,

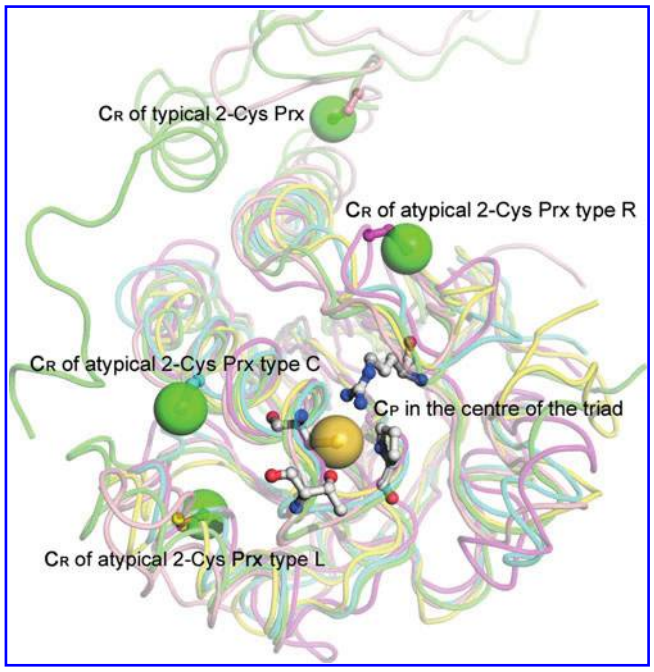

FIG. 5. Overlay of five representative peroxiredoxin subfamilies in their reduced form. 1-Cys (pdb: 1X0R) (85). Atypical 2-Cys type C (pdb: 2CX4). Atypical 2-Cys type R (pdb: 1HD2) (84) Atypical 2-Cys type L (pdb: 1PSQ). Typical 2-Cys (pdb: 2PN8). Triad of 2PN8 and resolving Cys (green) of the different subfamilies are shown (*ball and sticks*). Figure was obtained by using Pymol. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

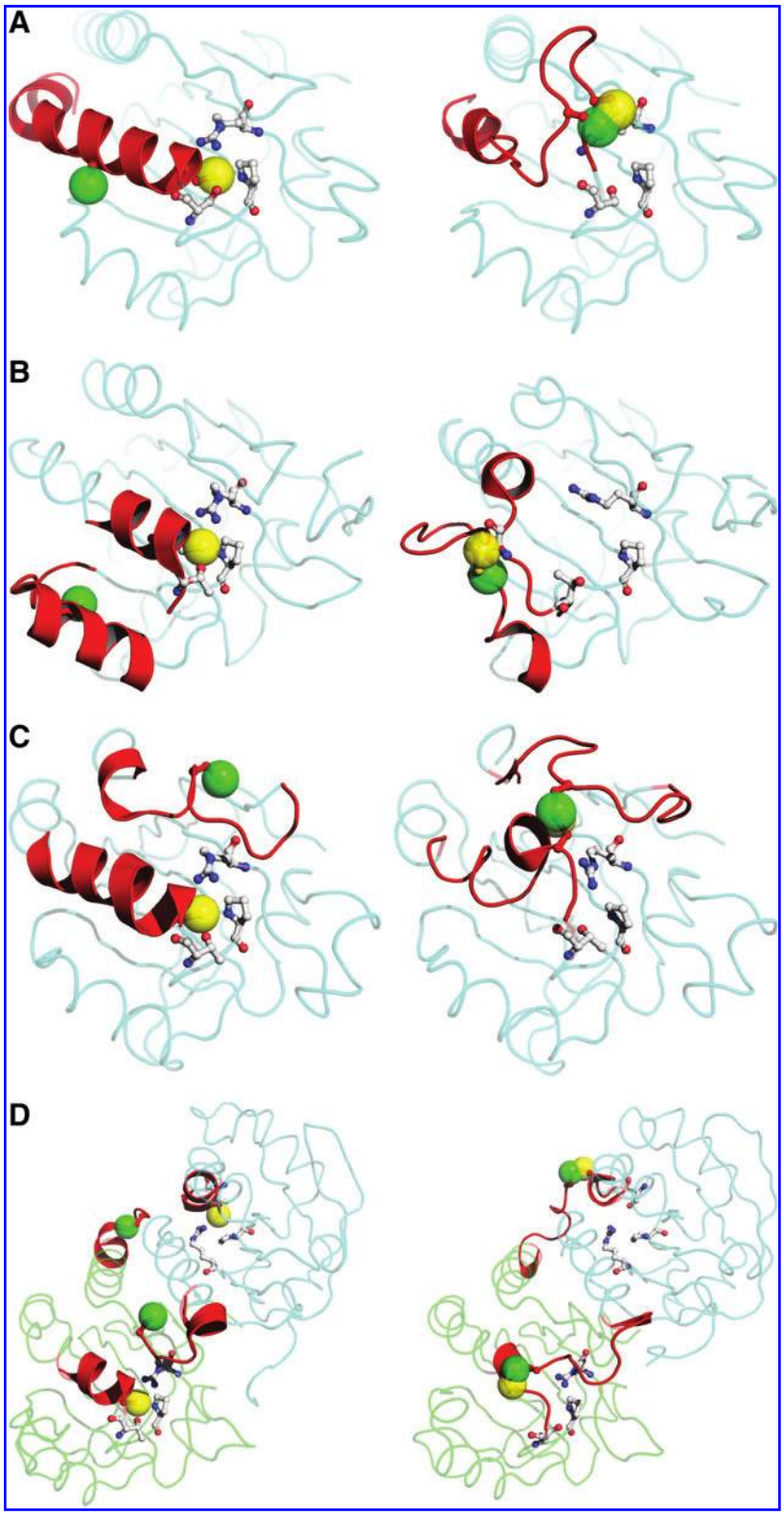

FIG. 6. Structural rearrangements on disulfide formation in 2-CysPrxs. Major conformational changes are marked in red; C_R, in green; and C_P, in yellow. Triad residues are shown (*ball and sticks*). *Left*, reduced forms; right, oxidized (disulfide) forms. The figures were obtained by using Pymol. **(A)** Atypical 2-CysPrx (C-type) from *Aeropyrum pernix* (pdb: 2CX4). The x-ray structure reports eight chains. On the left, the reduced form of chain E, and on the right, the oxidized form of chain C. **(B)** Atypical 2-Cys L-type Prx. On the left, the reduced form from *Streptococcus pneumoniae* (pdb: 1PSQ); and on the right, the oxidized form from *Escherichia coli* (pdb: 1QXH) (16). **(C)** Atypical 2-Cys R-type Prx (human Prx 5). Reduced form, pdb 1HD2 (20); oxidized form, pdb: 2VL9 (106). **(D)** Typical 2-CysPrx. *Left*, human Prx4 (pdb: 2PN8); right, Prx from *Amphibacillus xylanus* (pdb: 1WEO) (64). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

based on C_R position relative to the triad Arg in the amino acid sequence (17). In all examples, the C_P proved to be too remote from C_R for disulfide formation without unwinding of helices and extensive domain movements (Fig. 6). Interestingly, in the Prx family also "oxidized conformations" can be observed in reduced enzymes, which argues for an inherent flexibility of the chains harboring C_P and C_R. The x-ray analysis of the "bacterioferritin comigratory protein" from the *Aeropyrum pernix* (pdb: 2CX4), which is an atypical 2-CysPrx, reports on three different chains: E, B, and C (Fig. 7). Chain E shows the canonic triad of reduced Prx with the conserved Arg, and, in this case, an Ser perfectly coordinated with the C_P sulfur; chain B is equally reduced, but the Ser has completely lost contact with C_P, and the Arg approaches C_R; in chain C

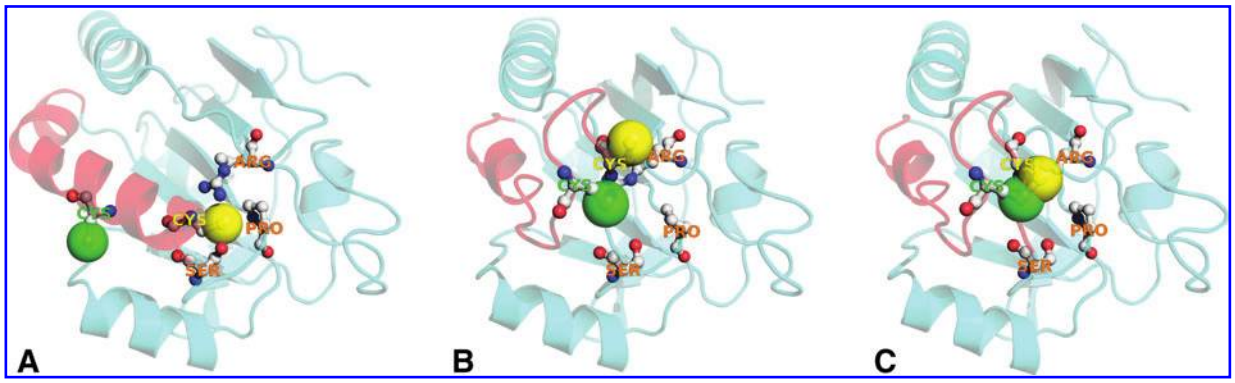

FIG. 7. Structural flexibility of an atypical 2-CysPrx. The x-ray analysis of the bacterioferritin comigratory protein from *Aeropyrum pernix* (pdb: 2CX4) reveals three different chain conformations; **(A)** and **(B)** represent reduced enzyme states, whereas chain **(C)** is in the oxidized disulfide form. The peroxidatic and resolving cysteines are shown in yellow and green, respectively. The reduced conformation **(A)** shows the catalytic cysteine embedded in the typical Prx triad, here with a functionally equivalent serine replacing the canonic threonine. In **(B)**, The peroxidatic triad is disrupted, although the enzyme is still reduced. Conformation **(C)** shows the intramolecular disulfide bridge characteristic of atypical 2-CysPrxs. The structures were obtained by using Pymol (http://www.pymol.org/). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

finally, the disulfide bridge of the oxidized enzyme is seen. Thus, peroxiredoxins may evidently display conformational dynamics similar to those demonstrated earlier for the glutathione peroxidase–type tryparedoxin peroxidase in Fig. 4. The functional relevance of the "transient" conformation "B" is unclear. Because of the disrupted catalytic triad, it is not suggestive of having peroxidatic activity, nor does it provide the ideal structure for the forthcoming reductive steps. The existence of this intermediate conformation, however, reveals that the seemingly unlikely conformational changes required for disulfide formation are programmed in the enzyme structures and do not strictly depend on previous oxidation of C_P.

Circumstantial evidence suggests that the susceptibility of Prxs to C_P overoxidation depends largely on how fast the structural changes that allow disulfide formation can proceed. In highly susceptible eukaryotic typical 2-CysPrxs, a YF motif in a C-terminal extension can interact with a GGLG motif near the C_P, thereby impeding the reaction of C_P with C_R (126). Deletion of the YF-containing domain in yeast thioredoxin peroxidase (68) and Prx2 and 3 of *Schistosoma mansoni* (102) abolished their pronounced peroxide sensitivity, whereas attachment of the Prx2 domain to the robust Prx1 of *S. mansoni* rendered the latter sensitive by allowing interaction with the GGLG motif (102). Direct evidence for the relevance of disulfide formation to peroxide-induced inactivation was obtained by mutating C_R to Ser in thioredoxin peroxidase of *Mycobacterium tuberculosis* (118). Whereas the wild-type enzyme was quite robust, the C_R mutant lost activity so fast that it appeared inactive in conventional testing, but proved to be as active as the wild-type protein in stopped-flow analysis. In parallel with the fast inactivation of the mutant, oxidation of C_P to the sulfinic form was detected by mass spectrometry.

Lack of C_P Protection: Failure of Evolution or Gain of Function?

Before embarking on the reductive part of thiol peroxidase catalysis, some emerging concepts of the possible biologic meaning of C_P overoxidation and the mechanisms of its prevention may be addressed. As outlined earlier, the oxidation equivalents of the C_P-SOH form of many CysGPxs and of the 2-CysPrxs are commonly "parked" in more stable disulfide bonds, before the reductive part of the catalytic cycle starts. In each of the paradigms, this intra- or intermolecular disulfide formation is associated with structural changes that appear complicated enough to cause a kinetic pause that often is so pronounced that the kinetic pattern is affected. Although the robust Prx1 of *S. mansoni* displays nonsaturable ping-pong kinetics (see later, Eq. 14), Prx2 and 3, in which the internal disulfide formation is impeded, show saturation kinetics (Eq. 15). Further, when the FY-containing tail of Prx2 was attached to Prx1, the latter behaved like Prx2 (102). Saturation kinetics implies that a monomolecular, substrate-independent reaction may become rate limiting. In Prxs, in which disulfide formation is impeded by interaction of the FY and GGLG motifs, the kinetic pause is evidently large enough, to make disulfide formation rate limiting. In respect to hydroperoxide detoxification, the kinetic pause thus imposed appears unfavorable; it reduces the maximal velocity to discrete values and allows destruction of the antioxidant device due to overoxidation. The wide phylogenetic conservation of this inherent break of the catalytic cycle therefore prompts the question of its possible physiological meaning.

Inactivation of Prx due to (reversible) overoxidation has been implicated in redox regulation (126). The "flood gate" theory assumes that, by inactivation of eukaryotic Prx, cellular hydroperoxides can transiently be increased to an extent that they can target exposed SH groups of less-reactive proteins in signaling cascades. The concept indeed appears attractive to explain adaptive responses to oxidative stress (*e.g.*, via the Nrf2/Keap1 and other stress-response systems) (113). It can, however, hardly account for signaling by physiologic levels of H_2O_2, as is increasingly reported for hormone-, growth factor-, or cytokine-triggered signaling (49, 51). In this context, target molecules in signaling cascades have to be postulated that can kinetically compete with abundant and highly efficient peroxidases (32, 44, 49). In higher organism, well-documented examples of highly redox-sensitive proteins that might fulfil this requirement in signaling machineries are scarce. The often-

quoted susceptible SH groups of tyrosine protein phosphatases do not react with H_2O_2 at rate constants faster than 43 $M^{-1}s^{-1}$ (8, 23, 108), which must be rated as noncompetitively slow. In contrast, the bacterial transcription factor OxyR is activated through direct oxidation by H_2O_2 at a rate constant near 10^5 $M^{-1}s^{-1}$ (4) (*i.e.*, well in the range of Prx and CysGPx efficiencies). Nevertheless, such exceptionally reactive SH groups are rare, with the exception of those of the thiol peroxidase active sites. Therefore, these peroxidases themselves should be ideal sensors and transducers in redox regulation.

A few examples of this kind of regulation have been documented. The yeast CysGPx Orp1, which is correctly characterized as a thioredoxin peroxidase, after oxidation of its active-site Cys to the sulfenic acid, can also become linked specifically to a particular SH group of the transcription factor Yap1, thereby oxidizing and activating the latter (22). Similarly, a 2-CysPrx can activate the stress kinase Sty1 (123), as well as the transcription factor Pap1 in *Saccharomyces pombe* (83). Mechanistically, these examples demonstrate that the kinetic pause imposed by the need of structural rearrangement in the regular catalytic cycle allows interacting with an alternate protein substrate, the sulfenic form attacking the SH of a specific partner protein instead of its own C_R. In this kind of redox regulation, unlike in the flood gate theory, overoxidation of C_P would arrest signaling and, thus, cause a refractory status. Considering the general need of structural changes in most of the CysGPxs and all 2-CysPrxs, it is tempting to speculate that physiologically meaningful interactions of these thiol peroxidases with alternate substrates are by far more frequent than currently suspected (32, 44, 83).

The Reductive Part of the Catalytic Cycles

In principle, the reductive part of the catalytic cycle of the thiol peroxidases is similar: the oxidized enzyme is stepwise reduced by two SH groups. The differences between the families and subfamilies consist of the mode of donor substrate binding and related specificities. The often pronounced donor substrate specificities imply that specific enzyme substrate complexes must be formed. However, the kinetic mechanism, which is common to these enzymes, suggests that such complexes are hard to analyze structurally, because they will decay fast by intracomplex reactions with product release. Therefore, present views are inevitably based on molecular modeling and docking calculations.

GPx1/GSH interaction

GPx1 of higher animals is a real *glutathione* peroxidase with pronounced specificity for GSH and, in this respect, likely represents a minority in the GPx family. Extensive specificity studies revealed that both carboxylic functions of GSH contribute to substrate binding (39, 111), and x-ray studies (27) as well as molecular modeling (5) suggested that the guanidino groups of Arg residues surrounding the active-site selenium are involved in GSH binding via electrostatic attraction, although the relative contributions of the four Arg residues that are close enough to be implicated may be questioned.

By chemical reasoning, the first reductive step is an attack of one GSH molecule on the oxidized selenium, be it an R-SO^- (Eq. 11) or the hypothetical Se-NH-R′ bond with NH-R′, meaning an amido nitrogen of the GPx itself (see earlier; Eq. 12)

$$\mathrm{GPx-SeO^- + H^+ + GSH \rightarrow GPx-Se-SG + H_2O} \quad (11)$$

$$\mathrm{GPx-Se-N-R' + GSH \rightarrow GPx-Se-SG + R'-NH_2)} \quad (12)$$

In both of the assumed starting positions, the selenium has to attack the GSH for selenylation. In the second step, a second molecule of GSH has to attack the selenyl-sulfide bond to regenerate the ground-state enzyme (Eq. 13).

$$\mathrm{GPx-Se-SG + GSH \rightarrow GPx-Se^- + H^+ + GSSG} \quad (13)$$

Reversibility of the last step is given in principle, but is considered negligible, because the symmetric disulfide GSSG is more stable than the polarized Se-S bond in the half-reduced catalytic intermediate GPx-Se-SG.

The x-ray structure of the tetrameric GPx1 shows the active-site Se atoms each surrounded by four Arg residues and an Lys of an adjacent subunit providing an additional positive charge for potential interaction with GSH (27, 71). An attempt to characterize GSH binding to GPx1 by means of cocrystallisation yielded low active-site occupancy, as expected from the kinetic mechanism, but suggested interactions of the carboxylic functions of GSH with R57 and R184 (residue numbers from a full-length bovine sequence) (71). In a molecular modeling approach (5), the same residues were implicated to bind GSH to bovine GPx1, although a different orientation of GSH appeared to be preferred. Moreover, the γ-Glu tail of GSH appeared to be attracted to the positive charges of R103 and K91′, the latter belonging to an adjacent subunit of the tetrameric enzyme. Thereby the sulfur of GSH was directed into an optimal position to react with the enzyme's oxidized Se, according to Eq. 11. The second GSH then appeared to be bound to the Se-glutathionylated intermediate by similar electrostatic interaction, whereby the second GSH had to compete with the already reacted one for R184, R103, and K91′ to cleave the selenyl-sulfide bond according to Eq. 13. GSH docking to human GPx1, as shown in Fig. 8, resembles the proposal of Epp *et al*. (27) in seeing the GSH in a bent conformation mainly squeezed between two Arg residues. Probably the rotational freedom of GSH provides different options for suitable accommodations at the active site.

The different approaches reveal that the reactions 11 or 12 and 13 involve the formation of enzyme–substrate complexes, which are primarily based on electrostatic interactions. They serve to bring the GSH thiols into productive positions, which can be achieved by different conformations and orientations of the GSH molecules. Conceivably, it requires time for the flexible GSH molecules to adopt a suitable position to react. Once this has been achieved, the reactions of the GSH thiols with the Se will proceed quickly, which complies with the observation that the complexes here implicated are kinetically silent. Therefore, the overall rate equation for GPx1 (Eq. 14)

$$[E_0]/v_0 = 1/k_{+1} \cdot [\mathrm{ROOH}] + 1/k'_{+2} \cdot [\mathrm{GSH}] \quad (14)$$

describes an enzyme-substitution mechanism without enzyme–substrate complexes, a type IVii mechanism in the systematic of Dalziel (19). In reality, however, only the first step, the enzyme oxidation, does not involve an enzyme–substrate complex, whereas the reductive steps (Eqs. 11–13) are mediated by

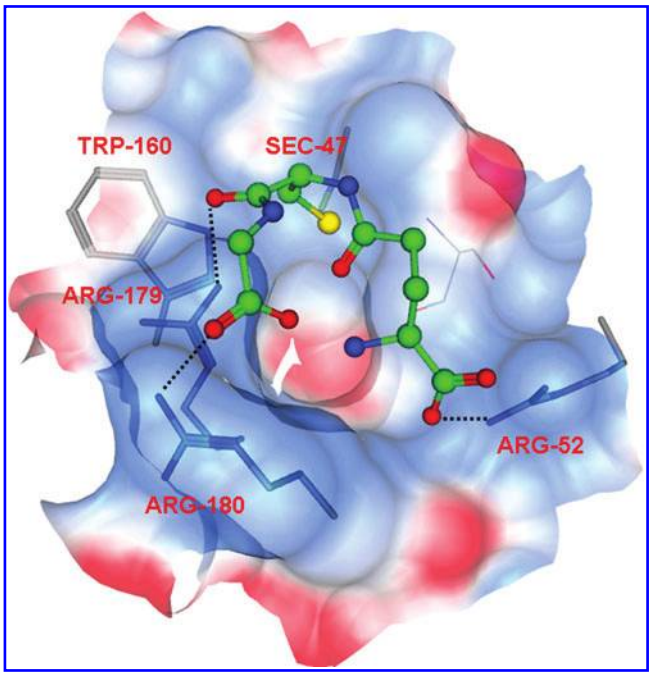

FIG. 8. Model of possible binding of first GSH to GPx1. One GSH molecule was docked to human GPx-1 (pdb: 2F8A). Original x-ray structure 2F8A is a Sec-to-Gly mutant; therefore, a Cys residue has been reconstructed in place of glycine before docking by using SCWRL4 (70). The Connolly electrostatic charge distribution surfaces of GPx-1 are highlighted (red negative and blue positive charges).The carboxylic functions of GSH are attracted to positively charged Arg residues and direct the sulfur of GSH toward the active-site Se, similar to that deduced from x-ray analysis of Epp *et al.* (27). For other residues implicated, see text and (5). The proteins and ligand preparation, as well as the docking procedure, were performed by using MOE (Molecular Operating Environment; http://www.chemcomp.com/software.htm). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

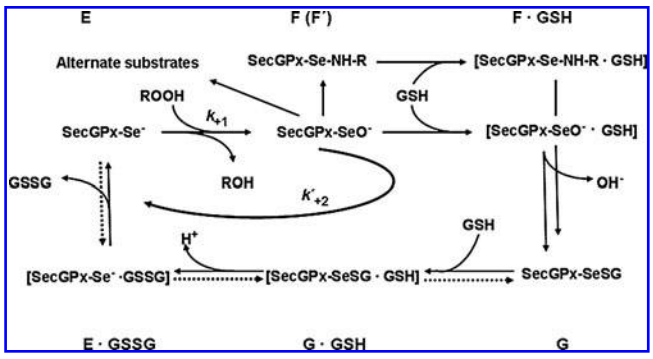

FIG. 9. Scheme of the catalytic cycle of a typical SecGPx. The ground-state enzyme E is oxidized to the selenenic acid intermediate F. Possibly, an intermediate F′ is formed (see text) before complex formation with the first GSH molecule. In the complex [F · GSH] a second intermediate G is formed, which again complexes GSH. Within the [G · GSH] complex, the product GSSG is formed with regeneration of E. The rate constants k_{+1} and k'_{+2} are those experimentally accessible, k'_{+2} being the net forward rate constant for the reductive part of the catalytic cycle.

enzyme–substrate complexes that are atypical in being formed less fast than they are processed. The complete catalytic cycle is schematically shown in Fig. 9.

Cosubstrate handling by other SecGPxs

The Arg and Lys residues discussed earlier for interaction of GPx1 with GSH are not consistently conserved in other SecGPxs, which points to a less-pronounced GSH specificity. The closest relative of GPx1 is GPx2, the gastrointestinal GPx. In this mammalian type of GPx, R57, 103, and 184, which are possibly responsible for GSH specificity, are conserved. We may therefore assume also that GPx2 is highly specific for GSH, although systematic specificity studies with the isolated enzyme are missing. In GPx3, the extracellular enzyme, only R103 and 185 are conserved. Expectedly, the GSH specificity is less strict. Discrete reactivity of GPx3 with thioredoxin and glutaredoxin has been reported (10, 77, 111). Also, k'_{+2}, the apparent net forward rate constant for the reductive steps, is significantly smaller than that for GPx1 (111). As outlined earlier, this finding reveals that GSH has even more difficulties in finding its appropriate orientation at the enzyme surface to react with the catalytic Se. In the monomeric GPx4, none of the residues implicated in GSH binding is reserved. It nevertheless is reduced by GSH with a k'_{+2} similar to that of GPx3, which has tentatively been explained by other basic residues that may functionally substitute for the canonic

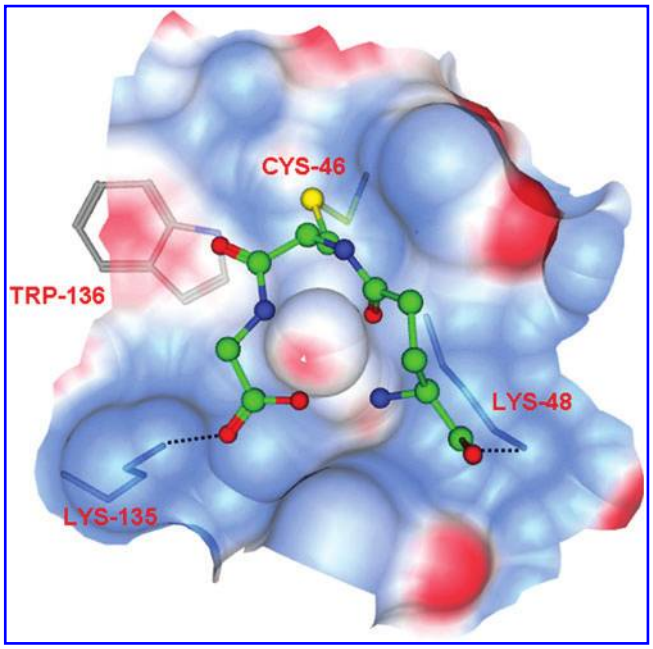

FIG. 10. Molecular docking of glutathione (GSH) bound to human GPx-4 (pdb: 2OBI). The Connolly electrostatic charge-distribution surfaces of GPX4 are highlighted (red, negative; and blue, positive charges). Lysine residues may substitute for the canonic Arg residues of GPx1 in GSH attraction. The proteins and ligand preparation, as well as the docking procedure, were performed by using MOE (Molecular Operating Environment; http://www.chemcomp.com/software.htm). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Args in GPx1 (78) (Fig. 10). However, the enzyme is exceptional not only in respect to its broad hydroperoxide specificity. It also accepts a wide variety of thiol substrates (98), including thiols of proteins such as chromatin (47) and the sperm mitochondria-associated cysteine-rich protein (75) with its characteristic double Cys motifs (53). In shortage of GSH, GPx 4 even reacts with exposed SH groups of itself (78, 103, 121). Mechanistically, this process is an alternate substrate self-inactivation. In biologic terms, it means that GPx4 can be a "moonlighting protein" that *S*-selenylates itself, thereby polymerizing, losing enzymatic activity and switching from a highly active peroxidase to a structural protein, a most unusual phenomenon that is, however, pivotal in spermiogenesis (30, 43, 53, 74, 78, 86, 103, 121).

Specificity switch in CysGPxs

Little is known about the specificities of mammalian CysGPxs, such as GPx5, 7, and 8, or the olfactory GPx6, which is a CysGPx in rodents, but an SecGPx in humans. The subfamily of CysGPx that prevails in bacteria, protozoa, fungi, green plants, and insects (74) and has an additional Cys in an N-terminal flexible loop intrigued with a donor substrate specificity and mechanism of action typical of 2-CysPrxs. By analogy, these proteins may be called 2-CysGPxs. They are the GPxs that form an internal disulfide bond, as mentioned earlier. They had widely been annotated as *glutathione* peroxidases according to sequence, but proved to accept redoxins preferentially as reductants (*i.e.*, proteins characterized by CxxC motifs).

This specificity switch in the GPx family was first discovered during futile attempts to detect any relevant glutathione peroxidase activity in a heterologous expression product of a *Plasmodium falciparum* gene (110) presumed to encode a GPx (45). Sequence comparison had disclosed that the *Plasmodium* enzyme was more similar to the less GSH-specific SecGPx4 than to any other GPx type, and in consequence, other thiol substrates were tested for sustaining a peroxidase function. Reasonable activity was obtained with various thioredoxins (Trx), that of *P. falciparum* yielding the best results. The net forward rate constant for the reduction by *Pf*Trx, k'_{+2}, was 5.4×10^4 $M^{-1}s^{-1}$, whereas that for GSH was just 20 $M^{-1}s^{-1}$. Moreover, a K_m for *Pf*Trx as low as 10 μM was obtained, whereas that for GSH was infinite. The important teachings of these findings are as follows:

1. The combination of an infinite K_m for GSH and a rate constant close to that for not catalyzed thiol/disulfide exchange reactions reveals that GSH can no longer be regarded as the physiologic substrate of the 2-CysGPx, but as an unspecific reductant.
2. The ability of 2-CysGPxs to form internal disulfide bridges, discovered later (22, 74), predestines them for reduction by the classic protein disulfide reductants, the thioredoxins or related CxxC proteins.
3. The interaction with Trx is specific, as evident from different activities with various Trx species.
4. The kinetic pattern is modified to an enzyme-substitution mechanism with real K_m and V_{max} values (type IVi mechanism according to Dalziel), revealing that, in contrast to the SecGPx mechanism, the decay of an enzyme–substrate complex has become rate limiting.

The overall rate equation, thus, is changed to

$$[E_0]/v_0 = 1/k_{cat} + 1/k_{+1} \cdot [ROOH] + 1/k'_{+2} \cdot [Trx(SH)_2] \quad (15)$$

The switch in the kinetic pattern is likely due to two factors: (a) complex formation between the disulfide form of the oxidized *Pf*GPx with a structurally rigid Trx is likely faster than between a typical GPx and the highly flexible GSH molecule; and (b) the attack of the exposed Trx thiol on the *Pf*GPx disulfide must be less efficient than that of GSH on the highly reactive selenenic acid form of a SecGPx. In consequence, the rate of complex formation exceeds the rate of downstream reactions, and the complex between $GPxS_2$ and $Trx(SH)_2$ can accumulate, as is generally assumed in the Michaelis–Menten theorem. Similar kinetics have meanwhile been found for the 2-CysGPx-type tryparedoxin peroxidases from *Trypanosoma brucei* (1) and *Leishmania major* (66), but not consistently observed with 2-CysGPxs; the thioredoxin peroxidase of *Drosophila* (77), and the tryparedoxin peroxidase II of *T. brucei* (104) did not display saturation kinetics. The rate constants for complex formation and intracomplex reaction for these enzymes are evidently so similar that tiny structural differences or even testing conditions can determine which one is faster (Fig. 11).

The driving forces leading to complex formation have not yet been analyzed experimentally. Complex modeling, however, suggests that primarily electrostatic attraction between

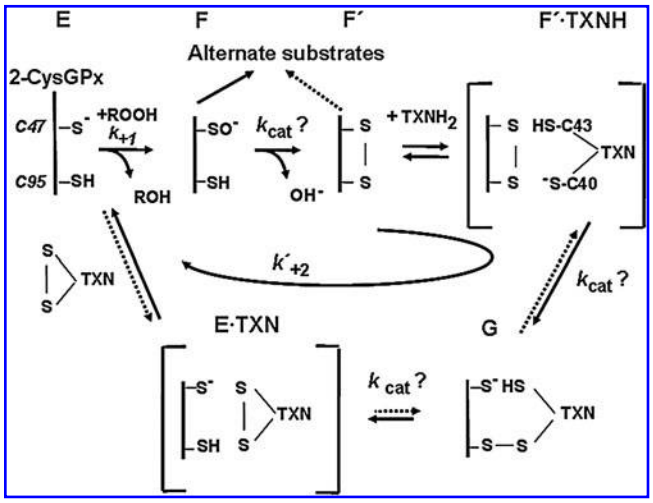

FIG. 11. Schematic representation of a 2-CysGPx reaction. Residue numbers correspond to those in Fig. 12. The ground-state enzyme E is oxidized to its sulfenic acid form F, which then reacts to the disulfide form F′. F′ forms a complex with a redoxin (here, tryparedoxin; TXN). Within this complex, thiol/disulfide exchange leads to a third intermediate G. By further thiol/disulfide exchange, G is transformed into the complex of E with oxidized TXN, from which E is regenerated with product release. k_{+1} is the rate constant for the oxidation of the enzyme by a particular hydroperoxide, whereas k'_{+2} is the net forward rate constant for the reduction by substrate, in the example chosen for the association of the GPx with TXN. If the overall reaction is limited by a maximal velocity, the corresponding k_{cat} is the rate constant of a monomolecular step, in *Tb*GPXIII, likely the step from $[F'TXNH_2]$ to G, but it can equally be the F to F′ transition, as discussed for Prxs susceptible to oxidative inactivation.

surface charges again accounts for orienting the substrates in a productive way. It may be stressed that neither the 2-CysGPxs nor their reducing protein substrates are designed for an extremely specific interaction with a single substrate according to the "lock and key" paradigm. As broad-spectrum peroxidases in their ground state, the 2-CysGPxs have to accept structurally diverse hydroperoxides; the C_P of their primary oxidation product is supposed to find its C_R in a remote loop as well as SH groups in other proteins; and the disulfide forms have to interact with proteins known to be pleiotropic, such as thioredoxins, (41, 61, 91), other redoxins (42, 55) or their substitutes in kinetoplasts, the tryparedoxins (12, 57, 69). How a complex between such multiple-task proteins may look is illustrated in the model of tryparedoxin/GPx interaction of *T. brucei,* which is based on established structures of the individual components (Fig. 12). The GPx shows its C_P (C47) engaged in the disulfide bond with C_R (C95), the former being completely turned out of the tetrad (C47, Q82, W137, and N138; compare Figs. 2 and 12). The C_P sulfur is hardly accessible, whereas now the C_R sulfur is surface exposed and can be attacked by the exposed Cys of the tryparedoxin (C40*). This constellation, which is favored by hydrogen bridges and salt bonds (see Fig. 12), allows a nucleophilic attack of C40* on C95 without any sterical infringements. What follows is a trivial thiol/disulfide exchange (Fig. 11): an electron pair moves from the tryparedoxin's exposed C40* to C95 (C_R), whereby a mixed disulfide as a third catalytic intermediate is formed. The release of oxidized tryparedoxin and reduced peroxidase is then achieved by electron-pair movement from C43* to C40* and further to C95, and the reduced C_P (C47) can return to its starting position in the tetrad.

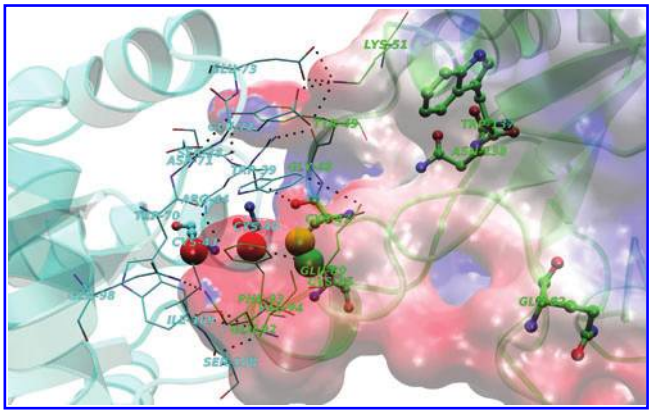

FIG. 12. Model of the interaction of the oxidized form of GPx-type tryparedoxin peroxidase *Tb*PxIII/, (pdb: 3DWV) (79) with reduced tryparedoxin reduced form (TXN, pdb: 1O85) (2) from *Trypanosoma brucei,* performed by ClusPro (18) protein–protein docking server. PxIII is shown with the Connolly electrostatic charge distribution (red negative and blue positive charges) calculated with Adaptive Poisson-Boltzmann Solver (APBS) (7), whereas TXN is shown in cartoon. Cysteines involved in catalysis and the amino acids of the tetrad are displayed (*ball and sticks*). Sulfur atoms of Cys^{40} and Cys^{43} of TXN are in red, whereas sulphur of peroxidatic Cys^{47} and resolving cysteines Cys^{95} forming a disulfide bridge are in yellow and green, respectively. Residues in contact between the two proteins are shown in lines and are Ser^{38}, Trp^{39}, Arg^{44}, Asp^{71}, Trp^{70}, Glu^{72}, Glu^{73}, Gln^{98}, Ser^{108}, Ile^{109} of TXN and Gly^{48}, Tyr^{49}, Lys^{51}, Glu^{89}, Glu^{92}, Phe^{93}, Val^{94} of the peroxidase. *Black dotted lines* indicate polar contacts (distance <4.0 Angstrom). Figure was constructed by using Pymol (http://www.pymol.org). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Reductive steps in Prx catalysis

The reaction scheme for 2-CysGPx (Fig. 11) can essentially be applied to all 2-CysPrxs, for atypical 2-CysPrxs just by changing the residue numbers. The only difference we have to make for the 2-CysPrxs is to let the C_P and the C_R react twice within a dimer in the transition from F to F′ and, of course, two redoxins are needed for the reduction. For the rare cases in which two monothiol substrate molecules are used instead of one redoxin-type dithiol substrate, the reaction scheme of Fig. 9 applies, when SecGPx is changed to Prx and Se to S. This scheme may also be valid for 1-CysPrx, the majority of which, though, is functionally not characterized.

Like the 2-CysGPxs, all 2-CysPrxs so far investigated are physiologically reduced by redoxins, be they thioredoxin, glutaredoxin, tryparedoxin, or homologous domains in bacterial AhpF. In the overwhelming number of 2-CysPrxs, disulfide formation between C_P and C_R proved to be a prerequisite for efficient reduction by these CxxC proteins, whereas (artificial) low-molecular-weight dithiols such as dithiothreitol are also accepted when the C_R is replaced by a nonfunctional residue (13, 92). The sulfenic form of a 2-CysPrx (F in Fig. 11) may thus be considered a dead-end intermediate, causing a block in physiologic Prx recycling that has to be "resolved" by a "resolving cysteine" C_R with formation of F′. The need of disulfide formation complies with the general reaction specificity of redoxins, which are widely known as protein disulfide reductants. Furthermore, available structures of oxidized 2-CysPrxs reveal that only the C_R appears exposed enough for nucleophilic attack by the exposed redoxin thiol (56, 92), and the redoxin/C_R interaction could be verified by mass spectrometry when a Prx-type tryparedoxin peroxidase was reacted with a tryparedoxin that had its coreacting Cys mutated to Ser (11). Finally, 1-CysPrxs, which cannot form the disulfide form, are not recycled by redoxins. Collectively, therefore, the disulfide forms of 2-CysPrxs (and GPxs) appear to present the ideal interface for redoxin attack.

Nevertheless, a direct reaction of the sulfenic forms with protein thiols, even of redoxins, is possible and may be of outstanding physiologic importance. A reduction of a mutant atypical 2-CysPrx of *M. tuberculosis* lacking C_R (*Mt*TPxC93S) by *Mt*TrxB was shown to be competitive with that of the wild-type enzyme, revealing that, in this case, the F′ formation is dispensable for recycling (118), although it was indispensable for prevention of overoxidation (see earlier). Certainly, the sulfenic form is the one that reacts with isolated protein SH groups, as in the already mentioned interaction with regulatory proteins (83, 123), and it is tempting to speculate that such reactions of the sulfenic forms could represent the actual biologic role of some Prxs, whereas the redoxin-mediated recycling is meant only to regenerate ground-state enzyme for renewed hydroperoxide sensing.

To review the donor-substrate specificities of Prxs is beyond the scope of this article. It may suffice to state that they are not particularly selective but by no means promiscuous

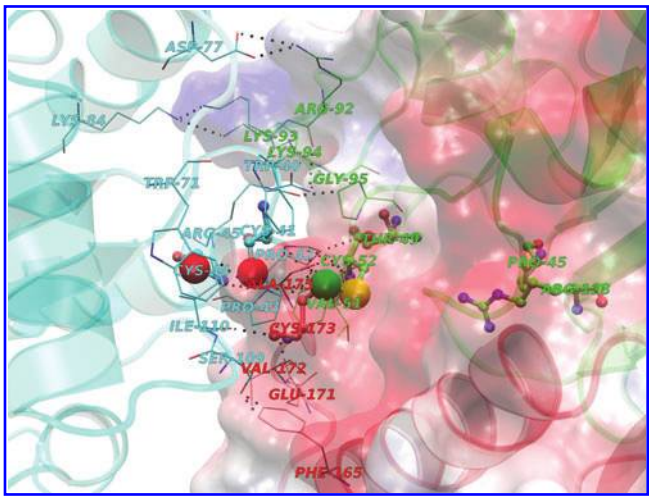

FIG. 13. Model of interaction of the typical 2-Cys Prx-type TXNPx with tryparedoxin (TXN), as modeled by Hofmann *et al.* (56). The two chains of TXNPx are shown with the Connolly electrostatic charge distribution (red, negative; and blue, positive charges) calculated with Adaptive Poisson-Boltzmann Solver (APBS) (7). The chain containing the peroxidatic cysteine (Cys^{52} with sulfur in yellow) is in green and is shown bonded by a disulfide bridge to the resolving cysteine (Cys^{173} with sulfur in green) of the second chain in red. Residues in contact between the two proteins are shown in lines and are Trp^{40}, Pro^{42}, Pro^{43}, Arg^{45}, Trp^{71}, Asp^{77}, Lys^{84}, Ser^{109}, and Ile^{110} of TXN, Val^{51}, Arg^{92}, Lys^{93}, Lys^{94}, Gly^{95} of the peroxiredoxin chain (in green) carrying the peroxidatic cysteine Cys^{52} and Val^{172}, Glu^{171}, and Phe^{165} of the peroxiredoxin chain (in red) carrying the resolving cysteine Cys^{173}. *Black dotted lines*, Polar contacts (distance <4.0 Å). Triad of the peroxiredoxin chain carrying the peroxidatic cysteine Cys^{52} (*ball and sticks*) sulfurs of Cys^{41} and Cys^{44} of TXN are shown in red. Figure was obtained by using Pymol (http://www.pymol.org/). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

[for reviews, see (24, 56, 58, 69, 92)]. The *Mt*TPx clearly distinguishes between different types of thioredoxins (58); the other extreme is a comparable reduction by Trx and GSH, as shown in Prx2 and 3 of *S. mansoni* (102). For 1-CysPrxs, low molecular thiols and protein SH groups are commonly suspected as co-substrates, but a variety of them were also shown to be reduced by ascorbate (81), thus challenging the dogma that all Prxs are thiol peroxidases. The structural features enabling the typical Prx/redoxin interaction are illustrated in Fig. 13. Despite the multiple contacts between the proteins, no typical "lock and key" situation is detectable, which complies with a discrete, but not stringent, substrate specificity.

Synopsis

The two families of thiol peroxidases catalyze the reduction of hydroperoxides by thiols, essentially by similar mechanisms. The active-site thiol or selenol is oxidized by ROOH without interim formation of an enzyme–substrate complex to a sulfenic acid or selenenic acid, respectively, and the latter is stepwise reduced by two R-SH equivalents (Figs. 9 and 11). The activation of the redox-active thiol (selenol) involves deprotonation, polarization, and proton shuttling to cleave the substrate's peroxyl bond. These basic principles applying to both families, the mechanistic details are distinct, as are the residues contributing to the chalcogen and substrate activation: a tetrad typically consisting of Sec(Cys), Asn, Gln, and Trp in the GPx family (Fig. 2), and a triad comprising Cys, Thr (Ser), and Arg in the Prx family (Fig. 1). The oxidation equivalents of the labile sulfenic or selenenic acid forms (F in reaction schemes) may be "parked" in more-stable derivatives F′, which contain intra- or intermolecular disulfide bridges in 2-CysGPxs and 2-CysPrxs, are structurally unknown in SecGPxs, and hypothetically mixed disulfides in 1-CysPrxs.

The reductive part of the catalysis starts with formation of a mixed disulfide (selenyl-sulfide) G between enzyme and a first reducing thiol, and the ground-state enzyme E is regenerated by further thiol/disulfide exchange in both families. Thiols, thus, are the typical substrates for both families, ascorbate as substrate of 1-CysPrxs so far being the only reported exception. Donor-substrate specificities vary between the families as well as within the families, and the warning not to annotate functions based on sequence homology (3) remains valid.

The residues determining specificity for a particular substrate may equally vary between enzymes of the same (compare Figs. 8 and 10) and distinct families (compare Figs. 12 and 13). As a general, but by no means strict, rule, SecGPxs may be suspected to prefer GSH or other monothiols as substrate, whereas 2-CysGPxs and 2-CysPrxs are overwhelmingly reduced by redoxin-type proteins.

The similarity of GPx and Prx mechanisms and specificities should, however, not be considered indicative of a common phylogenetic ancestor; it rather is an example of convergent evolution. This raises the question what the evolutionary pressure could have been that led to the realm of enzymes that seemingly meet the same task: reduction of hydroperoxides. Just focusing on the situation in mammals, we have to encounter eight GPx- and six Prx-type peroxidases on top of catalase, other heme peroxidases, and GSH-S-transferases doing similar jobs. Admittedly, most of the heme peroxidases are not in charge of H_2O_2 detoxification. It also appears conceivable that mammalian physiology was in need of an enzyme such as GPx4 that is specialized for removal of lipid hydroperoxides. Still, we have 13 thiol peroxidases plus catalase left to compete for H_2O_2. It is hard to believe that nature had to assure efficient antioxidant defense by creating a minimum of 14 redundant enzymes backing up each other. Rather, the risks of "oxidative stress," which for long dominated research on peroxidases, have detracted attention from other potential roles of these enzymes. Thiol peroxidases are indeed ideal candidates to "use" H_2O_2 or other hydroperoxides for redox regulation, differentiation, or other purposes, and their diversity is the key to lend specificity to the oxidant signaling molecule H_2O_2. Beyond that, their peculiar mechanism of action and the associated structural changes provide unusual chances to meet specific tasks that are completely distinct from fighting oxidative stress.

The enzyme-substitution mechanism, which is common to all thiol peroxidases, implies that we have to consider the different enzyme forms that constitute the catalytic cycle (E, F, F′, and G in Figs. 9 and 11) as proteins with distinct chemical characteristics, and there is ample evidence that additional distinct enzyme forms [*e.g.*, an E′ as a ground-state enzyme E with a distorted peroxidatic site (84) (see Figs. 4 and 7)], or an

overoxidized enzyme F″ may be implicated. Each of these forms offers a distinct substrate-interaction site that is by no means restricted to interaction with a substrate of the regular catalytic cycle. In the reaction schemes (Fig. 9 and 11), which equally apply to GPx and Prx, the arrow pointing from F to "alternate substrates" refers to the possibility that F, after having "sensed" H_2O_2, targets an SH group of another protein by its sulfenic (selenenic) acid function instead of a substrate thiol or the C_R. Documented examples are the polymerization of SecGPx4 in mammalian spermiogenesis (30, 121), the S-thiylation of Yap1 by Orp1 in *S. cerevisiae* (22), and the analogous reaction of the 2-CysPrx of *S. pombe* with Pap1 (83) and Sty1 (123). A similar de-branching from the catalytic cycle may be envisaged at the level of F′ of 2-CysGPx or Prx. These disulfide forms can, in principle, undergo thiol/disulfide exchange with protein thiols distinct from their regular substrates and, thus, may act as protein thiol–modifying agents. With GSH as co-substrate, G might transfer its glutathionyl residue to a protein SH instead of to a second GSH. Finally, the more or less pronounced specificity of the 2-Cys thiol peroxidases could account for the redox state of a particular redoxin that, in turn, can affect a regulatory circuit [*e.g.*, by redox-dependent non-covalent binding of Trx to ASK1 in humans (99) or termination of oxidant activation of Yap1 by reduced Trx in yeast (21, 22, 99)]. How widely all these potentials of the thiol peroxidases are exploited in nature remains a challenging question. Convincing answers have to be based on affinities and rate constants of the individual enzyme forms for the target proteins under consideration, relative to those for the regular recycling substrates (32, 44).

In short, some of the thiol peroxidases are certainly important for balancing a hydroperoxide challenge; others appeal as potential key players in the scenario of redox regulation. Depending on localization or association with supramolecular complexes, they may complement each other in sensing hydroperoxides and specifically transducing the oxidant signal to special targets. In this context, particularly the 2-CysGPxs and Prxs with their structural flexibility are emerging as multitask enzymes that are best suited to meet the specificity demand in redox signaling.

Acknowledgments

We thank Professor Stefano Moro, Molecular Modelling Section of the University of Padova, for invaluable technical support, and Birgit Hofmann, Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany, for providing data used to design Fig. 13. L.F. further acknowledges support by the COST Action CM0801 "New drugs for neglected diseases" and by the German Ministry of Science and Technology (BMBF; grant 0315220 A-D).

References

1. Alphey MS, König J, and Fairlamb AH. Structural and mechanistic insights into type II trypanosomatid tryparedoxin-dependent peroxidases. *Biochem J* 414: 375–381, 2008.
2. Alphey MS, Gabrielsen M, Micossi E, Leonard GA, McSweeney SM, Ravelli RB, Tetaud E, Fairlamb AH, Bond CS, and Hunter WN. Tryparedoxins from *Crithidia fasciculata* and *Trypanosoma brucei*: photoreduction of the redox disulfide using synchrotron radiation and evidence for a conformational switch implicated in function. *J Biol Chem* 278: 25919–25925, 2003.
3. Antosiewicz J, McCammon JA, and Gilson MK. Prediction of pH-dependent properties of proteins. *J Mol Biol* 238: 415–436, 1994.
4. Aslund F, Zheng M, Beckwith J, and Storz G. Regulation of the OxyR transcription factor by hydrogen peroxide and the cellular thiol-disulfide status. *Proc Natl Acad Sci U S A* 96: 6161–6165, 1999.
5. Aumann KD, Bedorf N, Brigelius-Flohé R, Schomburg D, and Flohé L. Glutathione peroxidase revisited: simulation of the catalytic cycle by computer-assisted molecular modelling. *Biomed Environ Sci* 10: 136–155, 1997.
6. Baker LM and Poole LB. Catalytic mechanism of thiol peroxidase from *Escherichia coli:* sulfenic acid formation and overoxidation of essential CYS61. *J Biol Chem* 278: 9203–9211, 2003.
7. Baker NA, Sept D, Joseph S, Holst MJ, and McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc Natl Acad Sci U S A* 98: 10037–10041, 2001.
8. Bindoli A, Fukuto JM, and Forman HJ. Thiol chemistry in peroxidase catalysis and redox signaling. *Antioxid Redox Signal* 10: 1549–1564, 2008.
9. Biteau B, Labarre J, and Toledano MB. ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. *Nature* 425: 980–984, 2003.
10. Björnstedt M, Xue J, Huang W, Akesson B, and Holmgren A. The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. *J Biol Chem* 269: 29382–29384, 1994.
11. Budde H, Flohé L, Hofmann B, and Nimtz M. Verification of the interaction of a tryparedoxin peroxidase with tryparedoxin by ESI-MS/MS. *Biol Chem* 384: 1305–1309, 2003.
12. Castro H and Tomas AM. Peroxidases of trypanosomatids. *Antioxid Redox Signal* 10: 1593–1606, 2008.
13. Chae HZ, Chung SJ, and Rhee SG. Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem* 269: 27670–27678, 1994.
14. Chae HZ, Robison K, Poole LB, Church G, Storz G, and Rhee SG. Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. *Proc Natl Acad Sci U S A* 91: 7017–7021, 1994.
15. Choi HJ, Kang SW, Yang CH, Rhee SG, and Ryu SE. Crystal structure of a novel human peroxidase enzyme at 2.0 A resolution. *Nat Struct Biol* 5: 400–406, 1998.
16. Choi J, Choi S, Choi J, Cha MK, Kim IH, and Shin W. 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, 2003.
17. Chon JK, Choi J, Kim SS, and Shin W. Classification of peroxiredoxin subfamilies using regular expressions. *Genom Informat* 3: 55–60, 2005.
18. Comeau SR, Kozakov D, Brenke R, Shen Y, Beglov D, and Vajda S. ClusPro: performance in CAPRI rounds 6-11 and the new server. *Proteins* 69: 781–785, 2007.
19. Dalziel K. Initial steady state velocities in the evaluation of enzyme-coenzyme-substrate reaction mechanisms. *Acta Chem Scand* 11: 1706–1723, 1957.
20. Declercq JP, Evrard C, Clippe A, Stricht DV, Bernard A, and Knoops B. Crystal structure of human peroxiredoxin 5,

a novel type of mammalian peroxiredoxin at 1.5 A resolution. *J Mol Biol* 311: 751–759, 2001.
21. Delaunay A, Isnard AD, and Toledano MB. H_2O_2 sensing through oxidation of the Yap1 transcription factor. *EMBO J* 19: 5157–5166, 2000.
22. Delaunay A, Pflieger D, Barrault MB, Vinh J, and Toledano MB. A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. *Cell* 111: 471–481, 2002.
23. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
24. Dietz KJ. The dual function of plant peroxiredoxins in antioxidant defence and redox signaling. *Subcell Biochem* 44: 267–294, 2007.
25. Dubuisson M, Vander Stricht D, Clippe A, Etienne F, Nauser T, Kissner R, Koppenol WH, Rees JF, and Knoops B. Human peroxiredoxin 5 is a peroxynitrite reductase. *FEBS Lett* 571: 161–165, 2004.
26. Ellis HR and Poole LB. Novel application of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole to identify cysteine sulfenic acid in the AhpC component of alkyl hydroperoxide reductase. *Biochemistry* 36: 15013–15018, 1997.
27. Epp O, Ladenstein R, and Wendel A. The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. *Eur J Biochem* 133: 51–69, 1983.
28. Flohé L. Glutathione peroxidase: fact and fiction. *Ciba Found Symp*: 95–122, 1978.
29. Flohé L. The selenoprotein glutathione peroxidase. In: *Glutathione: Chemical, Biochemical and Medical Aspects: Part A,* edited by Dolphin D, Poulson R, and Avramovic O. New York: John Wiley & Sons, 1989, pp. 644–731.
30. Flohé L. Selenium in mammalian spermiogenesis. *Biol Chem* 388: 987–995, 2007.
31. Flohé L. The labour pains of biochemical selenology: the history of selenoprotein biosynthesis. *Biochim Biophys Acta* 1790: 1389–1403, 2009.
32. Flohé L. Changing paradigms in thiology: from antioxidant defence towards redox regulation. *Methods Enzymol* 473: 1–39, 2010.
33. Flohé L and Brigelius-Flohé R. Selenoproteins of the glutathione system. In: *Selenium: Its Molecular Biology and Role in Human Health.* Edited by Hatfield DL. Boston: Kluwer Academic Publishers, 2001, pp. 157–178.
34. Flohé L and Brigelius-Flohé R. Selenoproteins of the glutathione system. In: *Selenium: Its Molecular Biology and Role in Human Health.* Edited by Hatfield D, Berry MS, and Gladyshev VN. New York: Springer, 2006, pp. 161–172.
35. Flohé L and Harris JR. Introduction: history of the peroxiredoxins and topical perspectives. *Subcell Biochem* 44: 1–25, 2007.
36. Flohé L and Ursini F. Peroxidase: a term of many meanings. *Antioxid Redox Signal* 10: 1485–1490, 2008.
37. Flohé L, Günzler WA, and Schock HH. Glutathione peroxidase: a selenoenzyme. *FEBS Lett* 32: 132–134, 1973.
38. Flohé L, Steinert P, Hecht HJ, and Hofmann B. Tryparedoxin and tryparedoxin peroxidase. *Methods Enzymol* 347: 244–258, 2002.
39. Flohé L, Günzler W, Jung G, Schaich E, and Schneider F. [Glutathione peroxidase. II. substrate specificity and inhibitory effects of substrate analogues]. *Hoppe Seylers Z Physiol Chem* 352: 159–169, 1971.
40. Flohé L, Budde H, Bruns K, Castro H, Clos J, Hofmann B, Kansal-Kalavar S, Krumme D, Menge U, Plank-Schumacher K, Sztajer H, Wissing J, Wylegalla C, and Hecht HJ. Tryparedoxin peroxidase of *Leishmania donovani*: molecular cloning, heterologous expression, specificity, and catalytic mechanism. *Arch Biochem Biophys* 397: 324–335, 2002.
41. Follmann H and Häberlein I. Thioredoxins: universal, yet specific thiol-disulfide redox cofactors. *Biofactors* 5: 147–156, 1995.
42. Fomenko DE and Gladyshev VN. Identity and functions of CxxC-derived motifs. *Biochemistry* 42: 11214–11225, 2003.
43. Foresta C, Flohé L, Garolla A, Roveri A, Ursini F, and Maiorino M. Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase. *Biol Reprod* 67: 967–971, 2002.
44. Forman HJ, Maiorino M, and Ursini F. Signaling functions of reactive oxygen species. *Biochemistry* 49: 835–842, 2010.
45. Gamain B, Langsley G, Fourmaux MN, Touzel JP, Camus D, Dive D, and Slomianny C. Molecular characterization of the glutathione peroxidase gene of the human malaria parasite *Plasmodium falciparum*. *Mol Biochem Parasitol* 78: 237–248, 1996.
46. Gladyshev VN. Selenoproteins and selenoproteomes. In: *Selenium: Its Molecular Biology and Role in Human Health.* Edited by Hatfield DL and Berry MJ. New York: Springer, 2006, pp. 99–110.
47. Godeas C, Tramer F, Micali F, Roveri A, Maiorino M, Nisii C, Sandri G, and Panfili E. Phospholipid hydroperoxide glutathione peroxidase (PHGPx) in rat testis nuclei is bound to chromatin. *Biochem Mol Med* 59: 118–124, 1996.
48. Goto K, Saiki T, and Okazaki R. Synthesis, structure, and reactions of stable selenenic acids bearing novel bridged calix(6)arene frameworks. *Phosphorus, Sulphur, Silicon* 136–138: 475–478, 1998.
49. Groeger G, Quiney C, and Cotter TG. Hydrogen peroxide as a cell-survival signaling molecule. *Antioxid Redox Signal* 11: 2655–2671, 2009.
50. Günzler WA, Vergin H, Müller I, and Flohé L. [Glutathione peroxidase VI: the reaction of glutahione peroxidase with various hydroperoxides]. *Hoppe Seylers Z Physiol Chem* 353: 1001–1004, 1972.
51. Hall A, Karplus PA, and Poole LB. Typical 2-Cys peroxiredoxins: structures, mechanisms and functions. *FEBS J* 276: 2469–2477, 2009.
52. Harris JR. Release of a macromolecular protein component from human erythrocyte ghosts. *Biochim Biophys Acta* 150: 534–537, 1968.
53. Hawthorne SK, Goodarzi G, Bagarova J, Gallant KE, Busanelli RR, Olend WJ, and Kleene KC. Comparative genomics of the sperm mitochondria-associated cysteine-rich protein gene. *Genomics* 87: 382–391, 2006.
54. Herbette S, Roeckel-Drevet P, and Drevet JR. Seleno-independent glutathione peroxidises: more than simple antioxidant scavengers. *FEBS J* 274: 2163–2180, 2007.
55. Hirota K, Matsui M, Murata M, Takashima Y, Cheng FS, Itoh T, Fukuda K, and Yodoi J. Nucleoredoxin, glutaredoxin, and thioredoxin differentially regulate NF-kappaB, AP-1, and CREB activation in HEK293 cells. *Biochem Biophys Res Commun* 274: 177–182, 2000.
56. Hofmann B, Hecht HJ, and Flohé L. Peroxiredoxins. *Biol Chem Hoppe Seyler* 383: 347–364, 2002.
57. Irigoin F, Cibils L, Comini MA, Wilkinson SR, Flohé L, and Radi R. Insights into the redox biology of *Trypanosoma cruzi*: trypanothione metabolism and oxidant detoxification. *Free Radic Biol Med* 45: 733–742, 2008.
58. Jaeger T. Peroxiredoxin systems in mycobacteria. *Subcell Biochem* 44: 207–217, 2007.

59. Jönsson TJ and Lowther WT. The peroxiredoxin repair proteins. *Subcell Biochem* 44: 115–141, 2007.
60. Jung BG, Lee KO, Lee SS, Chi YH, Jang HH, Kang SS, Lee K, Lim D, Yoon SC, Yun DJ, Inoue Y, Cho MJ, and Lee SY. A Chinese cabbage cDNA with high sequence identity to phospholipid hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. *J Biol Chem* 277: 12572–12578, 2002.
61. Kalinina EV, Chernov NN, and Saprin AN. Involvement of thio-, peroxi-, and glutaredoxins in cellular redox-dependent processes. *Biochemistry (Mosc)* 73: 1493–1510, 2008.
62. Karplus PA and Hall A. Structural survey of the peroxiredoxins. *Subcell Biochem* 44: 41–60, 2007.
63. Kim K, Kim IH, Lee KY, Rhee SG, and Stadtman ER. The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol/Fe(III)/O_2 mixed-function oxidation system. *J Biol Chem* 263: 4704–4711, 1988.
64. Kitano K, Kita A, Hakoshima T, Niimura Y, and Miki K. Crystal structure of decameric peroxiredoxin (AhpC) from *Amphibacillus xylanus*. *Proteins* 59: 644–647, 2005.
65. Koh CS, Didierjean C, Navrot N, Panjikar S, Mulliert G, Rouhier N, Jacquot JP, Aubry A, Shawkataly O, and Corbier C. Crystal structures of a poplar thioredoxin peroxidase that exhibits the structure of glutathione peroxidases: insights into redox-driven conformational changes. *J Mol Biol* 370: 512–529, 2007.
66. König J and Fairlamb AH. A comparative study of type I and type II tryparedoxin peroxidases in *Leishmania major*. *FEBS J* 274: 5643–5658, 2007.
67. König J, Lotte K, Plessow R, Brockhinke A, Baier M, and Dietz KJ. Reaction mechanism of plant 2-Cys peroxiredoxin: role of the C terminus and the quaternary structure. *J Biol Chem* 278: 24409–24420, 2003.
68. Koo KH, Lee S, Jeong SY, Kim ET, Kim HJ, Kim K, Song K, and Chae HZ. Regulation of thioredoxin peroxidase activity by C-terminal truncation. *Arch Biochem Biophys* 397: 312–318, 2002.
69. Krauth-Siegel LR, Comini MA, and Schlecker T. The trypanothione system. *Subcell Biochem* 44: 231–251, 2007.
70. Krivov GG, Shapovalov MV, and Dunbrack RL Jr. Improved prediction of protein side-chain conformations with SCWRL4. *Proteins* 77: 778–795, 2009.
71. Ladenstein R, Epp. O, Günzler WA, and Flohé L. Glutathione peroxidase on approval. In: *Life Chemistry Reports*, edited by Bannister JV and Michelson AM. New York: Harwood, 1986, pp. 37–55.
72. Little C, Olinescu R, Reid KG, and O'Brien PJ. Properties and regulation of glutathione peroxidase. *J Biol Chem* 245: 3632–3636, 1970.
73. Ma LH, Takanishi CL, and Wood MJ. Molecular mechanism of oxidative stress perception by the Orp1 protein. *J Biol Chem* 282: 31429–31436, 2007.
74. Maiorino M, Bosello V, Ursini F, Foresta C, Garolla A, Scapin M, Sztajer H, and Flohé L. Genetic variations of gpx-4 and male infertility in humans. *Biol Reprod* 68: 1134–1141, 2003.
75. Maiorino M, Roveri A, Benazzi L, Bosello V, Mauri P, Toppo S, Tosatto SC, and Ursini F. Functional interaction of phospholipid hydroperoxide glutathione peroxidase with sperm mitochondrion-associated cysteine-rich protein discloses the adjacent cysteine motif as a new substrate of the selenoperoxidase. *J Biol Chem* 280: 38395–38402, 2005.
76. Maiorino M, Aumann KD, Brigelius-Flohé R, Doria D, van den Heuvel J, McCarthy J, Roveri A, Ursini F, and Flohé L. Probing the presumed catalytic triad of selenium-containing peroxidases by mutational analysis of phospholipid hydroperoxide glutathione peroxidase (PHGPx). *Biol Chem Hoppe Seyler* 376: 651–660, 1995.
77. Maiorino M, Ursini F, Bosello V, Toppo S, Tosatto SC, Mauri P, Becker K, Roveri A, Bulato C, Benazzi L, De Palma A, and Flohé L. The thioredoxin specificity of *Drosophila* GPx: a paradigm for a peroxiredoxin-like mechanism of many glutathione peroxidases. *J Mol Biol* 365: 1033–1046, 2007.
78. Mauri P, Benazzi L, Flohé L, Maiorino M, Pietta PG, Pilawa S, Roveri A, and Ursini F. Versatility of selenium catalysis in PHGPx unraveled by LC/ESI-MS/MS. *Biol Chem* 384: 575–588, 2003.
79. Melchers J, Diechtierow M, Feher K, Sinning I, Tews I, Krauth-Siegel RL, and Muhle-Goll C. Structural basis for a distinct catalytic mechanism in *Trypanosoma brucei* tryparedoxin peroxidase. *J Biol Chem* 283: 30401–30411, 2008.
80. Mills GC. Hemoglobin catabolism, I: glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakown. *J Biol Chem* 229: 189–197, 1957.
81. Monteiro G, Horta BB, Pimenta DC, Augusto O, and Netto LE. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. *Proc Natl Acad Sci U S A* 104: 4886–4891, 2007.
82. Montemartini M, Kalisz HM, Hecht HJ, Steinert P, and Flohé L. Activation of active-site cysteine residues in the peroxiredoxin-type tryparedoxin peroxidase of *Crithidia fasciculata*. *Eur J Biochem* 264: 516–524, 1999.
83. Morgan BA and Veal EA. Functions of typical 2-Cys peroxiredoxins in yeast. *Subcell Biochem* 44: 253–265, 2007.
84. Muhle-Goll C, Fuller L, Ulrich AS, and Krauth-Siegel RL. The conserved Cys76 plays a crucial role for the conformation of reduced glutathione peroxidase-type tryparedoxin peroxidase. *FEBS Lett* 584: 1027–1032, 2010.
85. Nakamura T, Yamamoto T, Inoue T, Matsumura H, Kobayashi A, Hagihara Y, Uegaki K, Ataka M, Kai Y, and Ishikawa K. Crystal structure of thioredoxin peroxidase from aerobic hyperthermophilic archaeon *Aeropyrum pernix* K1. *Proteins* 62: 822–826, 2006.
86. Nayernia K, Diaconu M, Aumüller G, Wennemuth G, Schwandt I, Kleene K, Kuehn H, and Engel W. Phospholipid hydroperoxide glutathione peroxidase: expression pattern during testicular development in mouse and evolutionary conservation in spermatozoa. *Mol Reprod Dev* 67: 458–464, 2004.
87. Nelson KJ, Parsonage D, Hall A, Karplus PA, and Poole LB. Cysteine pK(a) values for the bacterial peroxiredoxin AhpC. *Biochemistry* 47: 12860–12868, 2008.
88. Netto LES, Chae HZ, Kang SW, Rhee SG, and Stadtman ER. Removal of hydrogen peroxide by thiol-specific antioxidant enzyme (TSA) is involved with its antioxidant properties: TSA possesses thiol peroxidase activity. *J Biol Chem* 271: 15315–15321, 1996.
89. Nogoceke E, Gommel DU, Kiess M, Kalisz HM, and Flohé L. A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. *Biol Chem* 378: 827–836, 1997.
90. Parsonage D, Karplus PA, and Poole LB. Substrate specificity and redox potential of AhpC, a bacterial peroxiredoxin. *Proc Natl Acad Sci U S A* 105: 8209–8214, 2008.
91. Perez-Jimenez R, Li J, Kosuri P, Sanchez-Romero I, Wiita AP, Rodriguez-Larrea D, Chueca A, Holmgren A, Miranda-Vizuete A, Becker K, Cho SH, Beckwith J, Gelhaye E, Jacquot JP, Gaucher EA, Sanchez-Ruiz JM, Berne BJ, and Fernandez JM. Diversity of chemical mechanisms in thioredoxin catalysis revealed by single-molecule force spectroscopy. *Nat Struct Mol Biol* 16: 890–896, 2009.

92. Poole LB. The catalytic mechanism of peroxiredoxins. *Subcell Biochem* 44: 61–81, 2007.
93. Poole LB and Claiborne A. The non-flavin redox center of the streptococcal NADH peroxidise, II: evidence for a stabilized cysteine-sulfenic acid. *J Biol Chem* 264: 12330–12338, 1989.
94. Poole LB and Ellis HR. Identification of cysteine sulfenic acid in AhpC of alkyl hydroperoxide reductase. *Methods Enzymol* 348: 122–136, 2002.
95. Ren B, Huang W, Akesson B, and Ladenstein R. The crystal structure of seleno-glutathione peroxidase from human plasma at 2.9 A resolution. *J Mol Biol* 268: 869–885, 1997.
96. Rocher C, Lalanne JL, and Chaudière J. Purification and properties of a recombinant sulfur analog of murine selenium-glutathione peroxidase. *Eur J Biochem* 205: 955–960, 1992.
97. Rotruck JT, Hoekstra WG, Pope AL, Ganther HE, Swanson A, and Hafemann D. Relationship of selenium to GSH peroxidase. *Fed Proc* 31: 691, 1972.
98. Roveri A, Maiorino M, Nisii C, and Ursini F. Purification and characterization of phospholipid hydroperoxide glutathione peroxidase from rat testis mitochondrial membranes. *Biochim Biophys Acta* 1208: 211–221, 1994.
99. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
100. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, and Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* 423: 769–773, 2003.
101. Sarma BK and Mugesh G. Antioxidant activity of the anti-inflammatory compound ebselen: a reversible cyclization pathway via selenenic and seleninic acid intermediates. *Chemistry* 14: 10603–10614, 2008.
102. Sayed AA and Williams DL. Biochemical characterization of 2-Cys peroxiredoxins from *Schistosoma mansoni*. *J Biol Chem* 279: 26159–26166, 2004.
103. Scheerer P, Borchert A, Krauss N, Wessner H, Gerth C, Höhne W, and Kühn H. Structural basis for catalytic activity and enzyme polymerization of phospholipid hydroperoxide glutathione peroxidase-4 (GPx4). *Biochemistry* 46: 9041–9049, 2007.
104. Schlecker T, Comini MA, Melchers J, Ruppert T, and Krauth-Siegel RL. Catalytic mechanism of the glutathione peroxidase-type tryparedoxin peroxidase of *Trypanosoma brucei*. *Biochem J* 405: 445–454, 2007.
105. Schremmer B, Manevich Y, Feinstein SI, and Fisher AB. Peroxiredoxins in the lung with emphasis on peroxiredoxin VI. *Subcell Biochem* 44: 317–344, 2007.
106. Smeets A, Marchand C, Linard D, Knoops B, and Declercq JP. The crystal structures of oxidized forms of human peroxiredoxin 5 with an intramolecular disulfide bond confirm the proposed enzymatic mechanism for atypical 2-Cys peroxiredoxins. *Arch Biochem Biophys* 477: 98–104, 2008.
107. Söhling B, Parther T, Rücknagel KP, Wagner MA, and Andreesen JR. A selenocysteine-containing peroxiredoxin from the strictly anaerobic organism *Eubacterium acidaminophilum*. *Biol Chem* 382: 979–986, 2001.
108. Sohn J and Rudolph J. Catalytic and chemical competence of regulation of cdc25 phosphatase by oxidation/reduction. *Biochemistry* 42: 10060–10070, 2003.
109. Splittgerber AG and Tappel AL. Steady state and pre-steady state kinetic properties of rat liver selenium-glutathione peroxidase. *J Biol Chem* 254: 9807–9813, 1979.
110. Sztajer H, Gamain B, Aumann KD, Slomianny C, Becker K, Brigelius-Flohé R, and Flohé L. The putative glutathione peroxidase gene of *Plasmodium falciparum* codes for a thioredoxin peroxidase. *J Biol Chem* 276: 7397–7403, 2001.
111. Takebe G, Yarimizu J, Saito Y, Hayashi T, Nakamura H, Yodoi J, Nagasawa S, and Takahashi K. A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein P. *J Biol Chem* 277: 41254–41258, 2002.
112. Tartaglia LA, Storz G, Brodsky MH, Lai A, and Ames BN. Alkyl hydroperoxide reductase from *Salmonella typhimurium*: sequence and homology to thioredoxin reductase and other flavoprotein disulfide oxidoreductases. *J Biol Chem* 265: 10535–10540, 1990.
113. Toledano MB. The guardian recruits cops: the p53-p21 axis delegates prosurvival duties to the Keap1-Nrf2 stress pathway. *Mol Cell* 34: 637–639, 2009.
114. Toppo S, Vanin S, Bosello V, and Tosatto SC. Evolutionary and structural insights into the multifaceted glutathione peroxidase (Gpx) superfamily. *Antioxid Redox Signal* 10: 1501–1514, 2008.
115. Toppo S, Flohé L, Ursini F, Vanin S, and Maiorino M. Catalytic mechanisms and specificities of glutathione peroxidases: variations of a basic scheme. *Biochim Biophys Acta* 1790: 1486–1500, 2009.
116. Tosatto SC, Bosello V, Fogolari F, Mauri P, Roveri A, Toppo S, Flohé L, Ursini F, and Maiorino M. The catalytic site of glutathione peroxidases. *Antioxid Redox Signal* 10: 1515–1526, 2008.
117. Trujillo M, Ferrer-Sueta G, Thomson L, Flohé L, and Radi R. Kinetics of peroxiredoxins and their role in the decomposition of peroxynitrite. *Subcell Biochem* 44: 83–113, 2007.
118. Trujillo M, Mauri P, Benazzi L, Comini M, De Palma A, Flohé L, Radi R, Stehr M, Singh M, Ursini F, and Jaeger T. The mycobacterial thioredoxin peroxidase can act as a one-cysteine peroxiredoxin. *J Biol Chem* 281: 20555–20566, 2006.
119. Ursini F, Maiorino M, and Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim Biophys Acta* 839: 62–70, 1985.
120. Ursini F, Maiorino M, Brigelius-Flohé R, Aumann KD, Roveri A, Schomburg D, and Flohé L. Diversity of glutathione peroxidases. *Methods Enzymol* 252: 38–53, 1995.
121. Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J, and Flohé L. Dual function of the selenoprotein PHGPx during sperm maturation. *Science* 285: 1393–1396, 1999.
122. van Montfort RL, Congreve M, Tisi D, Carr R, and Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* 423: 773–777, 2003.
123. Veal EA, Findlay VJ, Day AM, Bozonet SM, Evans JM, Quinn J, and Morgan BA. A 2-Cys peroxiredoxin regulates peroxide-induced oxidation and activation of a stress-activated MAP kinase. *Mol Cell* 15: 129–139, 2004.
124. Winterbourn CC and Metodiewa D. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radic Biol Med* 27: 322–328, 1999.
125. Woo HA, Chae HZ, Hwang SC, Yang KS, Kang SW, Kim K, and Rhee SG. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. *Science* 300: 653–656, 2003.
126. Wood ZA, Poole LB, and Karplus PA. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300: 650–653, 2003.
127. Wood ZA, Schröder E, Robin Harris J, and Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 28: 32–40, 2003.

Address correspondence to:
Prof. Leopold Flohé
MOLISA GmbH
Brennecke-Strasse 20
D-39118 Magdeburg
Germany

E-mail: l.flohe@t-online.de

Date of first submission to ARS Central, June 16, 2010; date of final revised submission, June 21, 2010; date of acceptance, July 18, 2010.

Abbreviations Used

C_P = peroxidatic cysteine
C_R = resolving cysteine
CysGPx = cysteine-containing glutathione peroxidase
Dm = *Drosophila melanogaster*
E_0 = total enzyme concentration
F = catalytic intermediate (oxidized)
G = catalytic intermediate (half reduced)
GPx(s) = glutathione peroxidase(s)
GPx1-8 = type 1-8 of glutathione peroxidase
GSH = reduced glutathione
GSSG = oxidized glutathione
Hs = *Homo sapiens*
Keap 1 = Kelch-like ECH-associated protein 1
M = moles/liter
Mt = *Mycobacterium tuberculosis*
NMR = nuclear magnetic resonance
Nrf2 = nuclear factor (erythroid-derived 2)-like 2
OxyR = regulator of hydrogen peroxide–inducible genes
Pf = *Plasmodium falciparum*
Prx(s) = peroxiredoxin(s)
Prx1-6 = types1–6 of peroxiredoxin
ROH = alcohol
ROOH = alkylhydroperoxide
s = second
Sec = selenocysteine
SecGPx = selenocysteine-containing glutathione peroxidase
Tb = *Trypanosoma brucei*
Trx = thioredoxin
$TrxS_2$ = oxidized thioredoxin
$Trx(SH)_2$ = reduced thioredoxin
TXN = tryparedoxin
TXNPx = tryparedoxin peroxidase
U_P = peroxidatic selenocysteine
v_0 = initial velocity

This article has been cited by:

1. Dr. Seung-Hyun Cho , Prof. Jean-Francois Collet . The many roles of the bacterial envelope reducing pathways. *Antioxidants & Redox Signaling* **0**:ja. . [Abstract] [Full Text PDF] [Full Text PDF with Links]
2. Marcel Deponte. 2012. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [CrossRef]
3. Aldwin Suryo Rahmanto, David I. Pattison, Michael J. Davies. 2012. Photo-oxidation-induced inactivation of the selenium-containing protective enzymes thioredoxin reductase and glutathione peroxidase. *Free Radical Biology and Medicine* **53**:6, 1308-1316. [CrossRef]
4. Carlos A. Tairum, Marcos A. de Oliveira, Bruno B. Horta, Fernando J. Zara, Luis E.S. Netto. 2012. Disulfide Biochemistry in 2-Cys Peroxiredoxin: Requirement of Glu50 and Arg146 for the Reduction of Yeast Tsa1 by Thioredoxin. *Journal of Molecular Biology* . [CrossRef]
5. David I. Pattison, Michael J. Davies, Clare L. Hawkins. 2012. Reactions and reactivity of myeloperoxidase-derived oxidants: Differential biological effects of hypochlorous and hypothiocyanous acids. *Free Radical Research* **46**:8, 975-995. [CrossRef]
6. Aurelie Nguyen Dinh Cat , Augusto C. Montezano , Dylan Burger , Rhian M. Touyz . Angiotensin II, NADPH Oxidase, and Redox Signaling in the Vasculature. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Seila Fernandez#Fernandez, Angeles Almeida, Juan P. Bolaños. 2012. Antioxidant and bioenergetic coupling between neurons and astrocytes. *Biochemical Journal* **443**:1, 3-11. [CrossRef]
8. Ho Zoon Chae , Hammou Oubrahim , Ji Won Park , Sue Goo Rhee , P. Boon Chock . 2012. Protein Glutathionylation in the Regulation of Peroxiredoxins: A Family of Thiol-Specific Peroxidases That Function As Antioxidants, Molecular Chaperones, and Signal Modulators. *Antioxidants & Redox Signaling* **16**:6, 506-523. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
9. Federico Fogolari, Alessandra Corazza, Stefano Toppo, Silvio C. E. Tosatto, Paolo Viglino, Fulvio Ursini, Gennaro Esposito. 2012. Studying Interactions by Molecular Dynamics Simulations at High Concentration. *Journal of Biomedicine and Biotechnology* **2012**, 1-9. [CrossRef]
10. Marlene Jimenez-Del-Rio, Carlos Velez-Pardo. 2012. The Bad, the Good, and the Ugly about Oxidative Stress. *Oxidative Medicine and Cellular Longevity* **2012**, 1-13. [CrossRef]
11. Stefania Gonfloni, Emiliano Maiani, Claudia Di Bartolomeo, Marc Diederich, Gianni Cesareni. 2012. Oxidative Stress, DNA Damage, and c-Abl Signaling: At the Crossroad in Neurodegenerative Diseases?. *International Journal of Cell Biology* **2012**, 1-7. [CrossRef]
12. James E. Trosko. 2011. Pre-Natal Epigenetic Influences on Acute and Chronic Diseases Later in Life, such as Cancer: Global Health Crises Resulting from a Collision of Biological and Cultural Evolution. *Journal of Food Science and Nutrition* **16**:4, 394-407. [CrossRef]
13. Francesco Galli, Andrea Battistoni, Roberto Gambari, Alfonso Pompella, Alessandra Bragonzi, Francesca Pilolli, Luigi Iuliano, Marta Piroddi, Maria Cristina Dechecchi, Giulio Cabrini. 2011. Oxidative stress and antioxidant therapy in cystic fibrosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [CrossRef]
14. Benjamin Selles, Martin Hugo, Madia Trujillo, Vaibhav Srivastava, Gunnar Wingsle, Jean-Pierre Jacquot, Rafael Radi, Nicolas Rouhier. 2011. Hydroperoxide and peroxynitrite reductase activity of poplar thioredoxin-dependent glutathione peroxidase 5 : kinetics, catalytic mechanism and oxidative inactivation. *Biochemical Journal* . [CrossRef]
15. Regina Brigelius-Flohé , Leopold Flohé . 2011. Basic Principles and Emerging Concepts in the Redox Control of Transcription Factors. *Antioxidants & Redox Signaling* **15**:8, 2335-2381. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
16. Ojia Skaff, David Pattison, Philip Morgan, Rushad Bachana, Vimal Jain, K. Priyadarsini, Michael Davies. 2011. Selenium-containing amino acids are major targets for myeloperoxidase-derived hypothiocyanous acid: determination of absolute rate constants and implications for biological damage. *Biochemical Journal* . [CrossRef]
17. Sue Goo Rhee , Hyun Ae Woo . 2011. Multiple Functions of Peroxiredoxins: Peroxidases, Sensors and Regulators of the Intracellular Messenger H2O2, and Protein Chaperones. *Antioxidants & Redox Signaling* **15**:3, 781-794. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
18. Goedele Roos, Joris Messens. 2011. Protein sulfenic acid formation: From cellular damage to redox regulation. *Free Radical Biology and Medicine* . [CrossRef]