Glutaredoxin-dependent Peroxiredoxin from Poplar

PROTEIN-PROTEIN INTERACTION AND CATALYTIC MECHANISM*

Received for publication, December 3, 2001, and in revised form, January 28, 2002
Published, JBC Papers in Press, February 6, 2002, DOI 10.1074/jbc.M111489200

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Recently, a poplar phloem peroxiredoxin (Prx) was found to accept both glutaredoxin (Grx) and thioredoxin (Trx) as proton donors. To investigate the catalytic mechanism of the Grx-dependent reduction of hydroperoxides catalyzed by Prx, a series of cysteine mutants was constructed. Mutation of the most N-terminal conserved cysteine of Prx (Cys-51) demonstrates that it is the catalytic one. The second cysteine (Cys-76) is not essential for peroxiredoxin activity because the C76A mutant retained ~25% of the wild type Prx activity. Only one cysteine of the Grx active site (Cys-27) is essential for peroxiredoxin catalysis, indicating that Grx can act in this reaction either via a dithiol or a monothiol pathway. The creation of covalent heterodimers between Prx and Grx mutants confirms that Prx Cys-51 and Grx Cys-27 are the two residues involved in the catalytic mechanism. The integration of a third cysteine in position 152 of the Prx, making it similar in sequence to the Trx-dependent human Prx V, resulted in a protein that had no detectable activity with Grx but kept activity with Trx. Based on these experimental results, a catalytic mechanism is proposed to explain the Grx- and Trx-dependent activities of poplar Prx.

Peroxiredoxins (Prxs) constitute a recently discovered family of non-heme peroxidases present in all organisms from prokaryotes to eukaryotes, and they catalyze the reduction of various hydroperoxides into the corresponding alcohol and water (1). Currently, these proteins are the subject of numerous studies because their function seems to be particularly important in the detoxification of reactive oxygen species that can cause serious damage to the nucleic acids, proteins, and lipids (2–4). Prx is also involved in the control of signal transduction by modulating the reactive oxygen species-mediated cellular responses and by regulating transcription factors (5–7).

All the Prx isoforms have in common a conserved catalytic cysteine, localized in the N-terminal part of the protein, that is converted into a sulfenic acid by hydroperoxides (8). This cysteine was demonstrated by site-directed mutagenesis to be essential for catalysis (9). Based mainly on the number of conserved cysteines, a classification has been proposed for the multiple existing Prxs. The 1-Cys Prxs mediate the reduction of H2O2 with the use of an unknown proton donor, which could be Trx for a mitochondrial 1-Cys Prx from Saccharomyces cerevisiae (10, 11). Recently, human cyclophilin A was identified as an electron donor to the mammalian Prx VI, the only mammalian 1-Cys Prx characterized, and also to all known mammalian Prxs (12). Among the Prxs with two conserved cysteines, at least three classes can be distinguished, according to the position of the cysteines. The first class, comprising mammalian Prx V, includes monomeric enzymes that form an intramolecular disulfide bridge as a reaction intermediate (13, 14). The second class is formed by the homologues of the bacterioferritin co-migratory protein, which are also shown to be monomeric enzymes with an intramolecular disulfide bridge in the oxidized state (15, 16). In this second class, the spacing between the two cysteines that are part of the disulfide bridge is considerably shorter, consistently containing 4 amino acids, instead of the ~100 amino acids for the first class. The third class, which includes mammalian Prx I to IV, consists of dimeric enzymes that form an intermolecular disulfide bridge between two identical subunits (14, 17). Despite these differences, the three types of 2-Cys Prx use Trx as a proton donor.

In a previous report, a poplar phloem Prx was characterized (18). This Prx is a small protein of 162 amino acids that contains only two cysteines in position 51 and 76. The primary sequence is quite different from that of the 1-Cys Prxs and most of the 2-Cys Prxs, especially because of the distance that separates the two cysteines. An unexpected finding was that this Prx could use Trx but also Grx as a proton donor for its catalysis (18). This Prx has been referred to as type C Prx, whereas the other 2-Cys Prxs, which use only Trx, were referred to as type A Prx, and 1-Cys Prxs were referred to as type B Prx. Among the biochemically well-characterized Prxs, one of the closest proteins is the mammalian Prx V. This enzyme also comprises 162 amino acids and displays 40% identity to poplar Prx at the amino acid level. A notable difference is the presence of an additional cysteine in position 152 that is linked together with Cys-51 to form an intramolecular disulfide bridge in Prx V (14). A Prx from Chinese cabbage, highly homologous to poplar Prx, was also shown to be a Trx-dependent enzyme, but no attempt was made to evaluate the potential for Grx as a proton donor in this work (19). To get a better understanding of the catalytic mechanism of this Grx-dependent Prx, cysteine mutants of Grx and Prx have been created by site-directed mutagenesis. Based on kinetic measurements and the creation of heterodimers, a new mechanism for the Grx-dependent Prx activity is proposed.

EXPERIMENTAL PROCEDURES

Materials—NADPH was obtained from Roche Molecular Biochemicals; diamide, β-mercaptoethanol, glutathione reductase, and reduced

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§ The abbreviations used are: Prx, peroxiredoxin; Grx, glutaredoxin; Trx, thioredoxin; WT, wild type; DTNB, 5,5'-dithiobis(nitrobenzoic acid).

This paper is available on line at http://www.jbc.org
glutathione were from Sigma. Dithiothreitol, isopropyl-1-thio-β-D-galactopyranoside, kanamycin, and ampicillin were from Fermentas.

**Cloning and Mutations of Grx and Prx**—The procedures for the isolation of the cDNAs and their subsequent cloning in expression plasmids are described in Refs. 18, 20, and 21. The mutagenesis of poplar Grx was effected as described (31). The Prx mutants C51A, C76A, and V152C were generated by PCR using the oligonucleotides shown below (NcoI and BamHI sites are underlined, mutagenic bases are in bold).

Cloning oligonucleotides were as follows: direct, 5'-GGGCGGGATCCCATCGGCCCAGATTGCTGGTGGTTG-3'; and reverse, 5'-GGGCGGGATCCCATCGGCCCAGATTGCTGGTGGTTG-3'. The mutated PCR products that contained the restriction sites were in turn cloned into the expression plasmid pET-3d, yielding the constructions pET Prx C51A, pET Prx C76A, and pET Prx V152C. The sequences of the recombinant plasmids were verified by sequencing.

**Expression and Purification of the Recombinant Proteins**—All procedures for the expression and purification of Arabidopsis thaliana NADPH Trx reductase, poplar Trx h1, and WT and mutant Grx are described elsewhere (20, 21, 23, 31). For the expression of Prx, the recombinant plasmids were used to transform Escherichia coli strain BL21(DE3), which was also co-transformed with the plasmid helper pSBET as described previously (18, 24). Because preliminary experiments indicated that Prx is susceptible to oxidation, all purification steps for the wild type and the three mutant proteins were done in the presence of 14 mM dithiothreitol or 20 mM mercaptoethanol.

**Thiol Content Titration**—The thiol content of each protein preparation was measured using the DTNB procedure. To eliminate the β-mercaptoethanol, 1 mg of each protein was incubated with 10% trichloroacetic acid for 30 min on ice. The mixture was then centrifuged for 15 min at 13,000 rpm, and the pellet was washed three times with 1 ml of 2% trichloroacetic acid. The pellet was resuspended in 100 mM Tris-HCl, pH 8, and 1 mM EDTA, and the protein concentration was determined by measuring the absorbance at 280 nm. SDS was then added to a final concentration of 1%, and the reaction was started by adding 100 μM DTNB. The reaction mixture was stored in the dark for 20 min, and the absorbance at 412 nm was measured. A second measurement was performed after a 30-min incubation in the dark. Both measurements gave identical results. Similar results were obtained when we used 80% acetone as a precipitant instead of trichloroacetic acid.

**Prx Activity Measurement**—The reduction of H$_2$O$_2$ by poplar Prx in the presence of the Trx or Grx system was followed spectrophotometrically using a Cary 50 spectrophotometer as described in Ref. 18. The activities were measured at a fixed concentration of Prx (2.5 μM) and at variable concentrations of Grx or Trx.

**Formation and Separation of the Heterodimers**—1.25 μg of Prx and Grx were mixed in the presence of TE buffer (30 mM Tris-HCl, pH 8, and 1 mM EDTA) to a final volume of 10 μl. This reaction mixture was incubated at room temperature for 2–3 min before the addition of 10 mM diamide. The mixture was incubated at room temperature for 20 min and then subjected to 14% SDS-PAGE in the presence of an equivalent volume of a nonreducing sample buffer (0.5 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, and bromphenol blue) (25). When needed, 10 mM dithiothreitol or 20–50 μM glutathione was added after incubation in the presence of diamide to reduce the covalent disulfide adducts between Prx and Grx.

**Western Blotting**—2 μl of the mixture described above were subjected to 14% SDS-PAGE before transfer. The immunodetection using the Immune Star Goat Anti Rabbit Detection Kit from Bio-Rad was performed as described in Ref. 18. Rabbit polyclonal antibodies against Trx, Grx, and Prx were purified onto affinity columns according to the procedure described for Prx antibodies in Ref. 18.

**RESULTS**

**Homologues of Poplar Prx among Other Species**—A previous study has indicated that poplar Prx differs from the other peroxiredoxins characterized thus far, essentially by the position of the conserved cysteine residues and by the overall length of the sequence (18). As detailed in the "Introduction," this class of new Prxs was called type C Prx, whereas 2-Cys Prxs were named type A Prx, and 1-Cys Prxs were called type B Prx. A number of sequences similar to type C poplar Prx have now appeared in the literature, and an amino acid comparison of some of the closest relatives is given in Fig. 1. Poplar Prx is strongly homologous to the other plant Prxs of its class (76%, 81%, and 82% amino acid identity with Oryza sativa, Brassica rapa, and A. thaliana, respectively) and also to the well-characterized PMP20 protein from Candida boidinii and to the human Prx V (42% and 40% amino acid identity, respectively) (Fig. 1). Nevertheless, an important difference is the presence of an additional cysteine in human Prx in position 152; the poplar and all other nonmammalian sequences do not possess
this additional cysteine. This particularity is examined here in terms of catalytic efficiency.

**Improvement of the Purification of Wild Type Prx and Grx**—In a previous work, we have shown that type C Prx could use both Grx and Trx as proton donors for catalysis. In this initial work, rather high concentrations of Trx or Grx were required in the *in vitro* measurement of peroxidase activity (18). Moreover, the specific activity of the enzyme remained low. We have improved this system in two respects: (i) the truncated form of poplar Grx, which was used in the earlier experiments, was replaced by a C-terminal-extended protein, which was both more stable and more active (20); and (ii) because we observed that Prx was susceptible to oxidation, its purification and subsequent storage were carried out in the presence of β-mercaptoethanol to avoid an irreversible oxidation of the catalytic cysteine. These changes resulted in a considerable enhancement of enzymatic activity, as shown in Table I. At a saturating Grx concentration (∼10 μM), the maximal specific activity of the enzyme is 8 μmol NADPH oxidized/min/mg protein. This value is similar to those reported for human peroxiredoxins and the *E. coli* bacterioferritin co-migratory protein (10, 14, 15). The specific activities recorded under the conditions of Table I suggest that Grx might be a better donor than Trx for poplar Prx. The maximal specific activity at a saturating Trx concentration estimated from the results in Fig. 3 is 3.55 μmol NADPH oxidized/min/mg protein, nearly in agreement with that proposal. However, these data should be treated with caution because the assays for Prx activity are indirect and involve the coupling of several components.

**Production of Mutant Proteins by Site-directed Mutagene-
sis**—Two monocysteinic mutants, called C27S and C30S, have been created to modify the active site of poplar Grx (31). In addition, the mutants C51A, C76A, and V152C have been engineered to explore the reactivity of poplar Prx. All these cysteine mutants were purified in the presence of β-mercaptoethanol to avoid undesired dimerizations. Fig. 2 shows that all Grx and Trx h1 preparations are highly homogeneous. As observed previously, all Prx preparations exhibited a protein doublet that cannot be eliminated by the addition of an excess of reductant (18). The reason for this behavior is unknown, but all biochemical and structural evidence gathered otherwise indicates that the protein is nevertheless highly homogeneous. Several reports in the literature indicate that the catalytic cysteine of peroxiredoxins can be transformed into sulfenic, sulfoxic, or sulfonic acids, and this has been proposed as the reason for the formation of apparent protein doublets (10). The titration results with DTNB do not really support such a hypothesis for the poplar protein (see below). Besides, as observed previously, if such a modification occurs, it is not the result of the purification procedure because similar protein doublets were observed after direct lysis of freshly harvested bacteria (data not shown).

**Thiol Titration of the Recombinant Proteins**—The thiol content of all protein preparations has been estimated using the DTNB method. All titrations were made on enzymes that were freed from reductant and after the addition of SDS (see “Experimental Procedures”). Consequently, all thiols are titrated, regardless of whether or not they were accessible in the native protein. A summary of these data is shown in Table II. For Grx, the results are quite simple; nearly two thiols are titrated for the reduced WT protein, and no SH group is present in the oxidized WT protein, suggesting that the two Cys residues are indeed linked in a disulfide bridge. The monocysteinic mutants of Grx give values around 1 SH/mol protein, as expected. For Prx, the results are more complex to analyze and depend on whether the protein was isolated in the presence or absence of a reductant. When prepared under nonreducing conditions, the WT Prx shows nearly 2 SH/enzyme monomer, and it appears that the C51A and C76A mutants are partially oxidized because values lower than 1 SH/mol were recorded. The V152C mutant gives a value of 2.6 SH/mol, nearly in agreement with the expected 3 SH/mol. When the Prx preparations were made in the presence of a monothiol reductant β-mercaptoethanol, very contrasting results were obtained. The C51A mutant showed nearly 1 SH/mol, but all other preparations had a thiol content that was reduced by about 1 SH group compared with the expected theoretical value. We interpret this as the result of a likely interaction between the thiol group of Cys-51 and β-mercaptoethanol that can give rise to a mixed disulfide that cannot be titrated with DTNB. As Prx preparation treated with the dithiol reductant dithiothreitol titrate as the enzyme prepared under oxidizing conditions, this formation of mixed disulfide is postulated to be specific for β-mercaptoethanol. Such a behavior has already been described for the bovine 1-Cys peroxiredoxin (26). Alternatively, because the thiol titrations are not always in perfect agreement with the theoretical values, this could also indicate that a portion of the peroxiredoxin molecules is in a denatured oxidized form with an internal disulfide bridge as described by Kang et al. for human 1-Cys Prx (10) or that a portion of the catalytic cysteine is in an oxidized form.

**Peroxidase Activity of the Mutant Peroxiredoxins**—The catalytic capacity of all Prx mutants has been evaluated in the presence of the various engineered Grxs as proton donors. This has been tested in a coupled enzymatic reaction where the peroxiredoxin-catalyzed conversion of H₂O₂ is linked to NADPH oxidation via glutathione reductase, glutathione, and glutaredoxin. We have shown previously, using the 2-hydroethyldisulfide and dehydroascorbate reduction tests, that Cys-27 is the catalytic residue of Grx, and Cys-30 is generally dispensable in those reactions (31). Fig. 3 shows the H₂O₂-dependent NADPH oxidizing activities of the various engineered peroxiredoxins as a function of Grx concentrations ranging from 0.5 to 20 μM. Only four combinations were found to promote catalysis. The best reactivity was obtained when WT Prx was associated with WT Grx. The mutation of Cys-30 of Grx (C30S) has little effect because the rate of catalysis with this mutant is nearly identical to the one obtained with WT Grx. The other associations that are catalytically competent are those between Prx C76A and Grx WT or

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**Table I**

| Initial Grx | Improved Grx | Trx |
|-------------|--------------|-----|
| Pox oxidizing conditions | I.d. | 3.55 |
| Pox reducing conditions | I.d. | 4.02 |

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Fig. 2. Analysis of purified recombinant proteins by SDS-PAGE. 14% SDS-PAGE under reducing conditions showing recombinant Trx, Grx, and Prx samples. Lane a, WT Trx h1; lane b, WT Prx; lane c, Prx C51A; lane d, Prx C76A; lane e, Prx V152C; lane f, WT Grx; lane g, Grx C27S; lane h, Grx C30S.
TABLE II
Thiol content of Grx and Prx purified under oxidizing or reducing conditions

|                     | Oxidizing conditions | Reducing conditions |
|---------------------|----------------------|---------------------|
| Grx WT              | 0.05                 | 1.67                |
| Grx C27S            | n.d.                 | 0.88                |
| Grx C30S            | 0.93                 | 0.9                 |
| Prx WT              | 1.7                  | 0.9                 |
| Prx C51A            | 0.28                 | 0.87                |
| Prx C76A            | 0.33                 | 0.19                |
| Prx V152C           | 2.6                  | 1.4                 |

Fig. 3. Grx-dependent Prx activities. H₂O₂ consumption by poplar Prx in the presence of the Grx system was followed in the coupled reaction system by NADPH oxidation/min as a function of Grx concentration. The WT and mutant Prx were associated in this test with each of the Grxs: WT Prx associated with WT Grx (○) or Grx C30S (□), Prx C76A associated with WT Grx (□) or Grx C30S (□). Prx C51A associated with any Grx, Prx V152C associated with any Grx, WT Prx associated with Grx C27S, and Prx C76A associated with Grx C27S (X).

C30S. However, the activities of the C76S mutant are reduced by approximately 75% compared with those recorded with WT Prx. Another observation is that the Prx C51A and V152C enzymes are completely inactive with Grx as a proton donor. A last piece of information is that the C27S mutant of Grx is unable to promote catalysis for all Prxs tested.

Fig. 4 presents the catalytic activity of the Prx enzymes, in the presence of WT Trx as a proton donor. In this case, the Grx generating system was replaced by the Trx system, which is composed of the A. thaliana NADPH thioredoxin reductase and poplar Trx h1 (18). The reduction of poplar Trx by the Arabidopsis NADPH thioredoxin reductase was previously shown to be functional using the DTNB reduction test (21). In general, similar results were obtained with the Trx system: the WT Prx was the most active catalyst, the mutation C51A abolished catalysis, and C76A had a depressing effect. The most striking difference is that the V152C protein, which was inactive with Grx as a proton donor, was catalytically active with Trx (Fig. 4, ♦). No saturation of Prx V152C enzyme activity could be recorded with Trx up to concentrations of 50 μM (data not shown). From the data in Figs. 3 and 4, Kᵣ values can be determined to characterize interactions between Prx and Grx or Trx. Similar values were obtained for Grx and Trx (2.5 and 3 μM, respectively). These values are in good agreement with other parameters published in the literature for Trx (14).

Heterodimer Formation—The mutation of the cysteine that is potentially involved in the breaking of the mixed disulfide intermediates stabilizes the heterodimers between Trx or Grx and their interacting partners (27). We have taken advantage of that property to create heterodimers between the peroxiredoxin and glutaredoxin molecules. The covalent heterodimer formation was greatly improved using the oxidant diamide. The four different Prx preparations (WT, C51A, C76A, and V152C) were incubated with three different versions of the Grx (WT, C27S, and C30S). Fig. 5 shows the results of these associations. In all assays, the Grx polypeptide is present with an apparent molecular mass of 15 kDa and the Prx is present with the usual doublet at ~18 kDa. By running the proteins individually and based on the molecular mass determinations, we could assess the additional polypeptides as follows: the 30-kDa polypeptide is likely a Grx dimer, the 33-kDa polypeptide a heterodimer, and the 37-kDa polypeptide is a Prx dimer. Fig. 5 shows that a heterodimer is efficiently created when using Grx C30S together with either WT Prx (lane c) or Prx C76A (lane i) and is created much less efficiently with Prx V152C (lane k).

The C27S mutant was also able to create heterodimers with the same Prx preparations, but with a much reduced efficiency (lanes b, h, and k). Strikingly, Prx C51A is the only preparation that does not form any dimer with all Grx (lanes d–f), indicating that this residue is an essential partner in the redox interaction. The WT Grx is unable to create any stable association with any Prx (lanes a, d, g, and j). Finally, a major additional polypeptide of ~48 kDa is present in the interaction between Grx C30S and Prx V152C. This high molecular mass could correspond to a Prx linked with two Grx molecules.

The nature of these polypeptides was confirmed by Western blotting experiments. As shown in Fig. 6, anti-Grx antibodies do not react with Prx (lane f) and vice versa (lane g). This experiment reveals that the band at 30 kDa is indeed a Grx dimer (lane d), the one at 33 kDa is a heterodimer between Grx and Prx (lanes e and h), and the one at 37 kDa is a dimer of Prx (lane i).

A further confirmation of the nature of the heterodimer is shown in Fig. 7. The heterodimer generated in the presence of diamide can be reduced in the presence of excess dithiothreitol or reduced glutathione (Fig. 7, lanes b and d). The disappearance of the heterodimer polypeptide after reduction confirms that the two chains are indeed linked together via a disulfide bond.

DISCUSSION

The poplar Prx described here is the only characterized Prx that accepts both Grx and Trx as an electron donor, whereas mammalian peroxiredoxins were shown to use only Trx or an...
unidentified donor as a proton source (14, 18). The poplar enzyme studied in this report is clearly a homologue of human Prx V (14). Both proteins are monomeric and contain 162 amino acids. The major difference between the two enzymes is their cysteine residue is present only in the mammalian sequences and not in the plant homologues of poplar Prx or the C. boidinii sequence (see Fig. 1). Mutagenesis studies on human Prx V have shown that this protein belongs to a new class of Prxs (14). Site-directed mutagenesis has clearly shown that Cys-47 is the catalytic residue on this protein, similar to all other peroxiredoxins. The catalytic mechanism of human Prx V necessitates Cys-47 alone with dithiothreitol as a donor. Moreover, the thiol titrations do not support the presence of a disulfide bridge between Cys-51 and Cys-76 on this protein. We postulate thus that the decrease in catalytic efficiency of the C76A mutant is linked to a modification of the microenvironment of the catalytic cysteine because of the introduction of the alanine residue in place of Cys-76. It is noteworthy that a slightly different mutation has been made in the case of human Prx V (C72S instead of C76A here), and this might be a reason for the different behavior of the plant mutant. Another line of evidence that Cys-76 is indeed not linked by a disulfide bridge unless there is an important conformational change.

The mutagenesis experiments presented here also provide experimental evidence for the Grx-dependent catalytic mechanism of poplar Prx. From the heterodimer experiments, it is clear that the two residues that interact between Prx and Grx are Cys-51 and Cys-27, respectively. The absence of het-
erodimer formation in the presence of the WT Grx is a clear indication that Cys-30 of Grx is the backup cysteine responsible for the breaking of the heterodisulfide. A scheme for the reaction of Prx in the presence of WT Grx is shown in Fig. 8A. As for the other Prx, the catalysis proceeds through the formation of a sulfenic acid on Cys-51. The latter is reduced, and a disulfide bond is formed with Cys-27 of Grx with the release of one water molecule. The heterodisulfide is then broken by Cys-30 of Grx, and the reoxidized Grx molecule is regenerated by reduced molecule. The heterodisulfide is then broken by Cys-30 of Grx, the other Prx, the catalysis proceeds through the formation of an intramolecular disulfide bridge in the human enzyme, and its absence in the plant protein. Our results clearly indicate that WT Prx and mutant C76A can both use Trx and Grx as donors. Both these enzymes, unlike the mammalian protein, lack Cys-152. When we engineered the plant protein and produced the V152C mutant that is similar to the mammalian protein, this enzyme conserved activity with Trx as a donor but lost its capacity to use Grx as a reductant. The maximal activity obtained with the Prx V152C mutant is similar to that described for human Prx V. The poplar Prx has thus acquired a behavior similar to that of the mammalian protein. Because we could observe that Grx C27S is still able to bind to the V152C mutant but produces higher molecular mass complexes that are likely to contain two Grx molecules, possibly by crystallizing the covalent complexes and solving its three-dimensional structure. The successful creation of disulfide-linked heterodimers between WT Prx and Trx h1 C42S should also help in this respect (data not shown). The solution of the three-dimensional structure of poplar Prx should also help us to understand why this protein accepts two donors, but human Prx V does not. The results obtained in this study suggest that Cys-152 is a critical residue for this property, but other domains of the proteins may be equally important as well.

Acknowledgment—We thank Dr. Francis Martin for valuable help and discussions.

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Brassica and Arabidopsis proteins should be able to use Grx as a donor as well. Whether this type is restricted to plants is still an open question.

Because the mutagenesis results suggest that the interaction sites for Grx and Trx are different on the plant Prx, it will be very interesting to map the areas of contact between the molecules, possibly by crystallizing the covalent complexes and solving its three-dimensional structure. The successful creation of disulfide-linked heterodimers between WT Prx and Trx h1 C42S should also help in this respect (data not shown). The solution of the three-dimensional structure of poplar Prx should also help us to understand why this protein accepts two donors, but human Prx V does not. The results obtained in this study suggest that Cys-152 is a critical residue for this property, but other domains of the proteins may be equally important as well.
Glutaredoxin-dependent Peroxiredoxin from Poplar: PROTEIN-PROTEIN INTERACTION AND CATALYTIC MECHANISM
Nicolas Rouhier, Eric Gelhaye and Jean Pierre Jacquot

J. Biol. Chem. 2002, 277:13609-13614.
doi: 10.1074/jbc.M111489200 originally published online February 6, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M111489200

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