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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 42, pp. 31736–31742, October 20, 2006

Glutathionylation Induces the Dissociation of 1-Cys D-peroxiredoxin Non-covalent Homodimer*[S]

Received for publication, March 8, 2006, and in revised form, June 9, 2006 Published, JBC Papers in Press, August 17, 2006, DOI 10.1074/jbc.M602188200

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1-Cys peroxiredoxins (1-Cys Prxs) are antioxidant enzymes that catalyze the reduction of hydroperoxides into alcohols using a strictly conserved cysteine. 1-Cys B-Prxs, homologous to human PrxVI, were recently shown to be reactivated by glutathione *S*-transferase (GST) π via the formation of a GST-Prx heterodimer and Prx glutathionylation. In contrast, 1-Cys D-Prxs, homologous to human PrxV, are reactivated by the glutaredoxin-glutathione system through an unknown mechanism. To investigate the mechanistic events that mediate the 1-Cys D-Prx regeneration, interaction of the Prx with glutathione was studied by mass spectrometry and NMR. This work reveals that the Prx can be glutathionylated on its active site cysteine. Evidences are reported that the glutathionylation of 1-Cys D-Prx induces the dissociation of the Prx non-covalent homodimer, which can be recovered by reduction with dithiothreitol. This work demonstrates for the first time the existence of a redox-dependent dimer-monomer switch in the Prx family, similar to the decamer-dimer switch for the 2-Cys Prxs.

Peroxiredoxins (Prxs)[3] represent a novel family of peroxidases that reduce hydrogen peroxide and hydroperoxides to water and alcohols using a strictly conserved cysteine (for review, see Refs. 1 and 2). When reducing the peroxide substrate, the catalytic cysteine of the Prx is oxidized to a cysteine-sulfenic acid (3, 4). The sulfenic acid is then reduced by a thiol-containing electron donor (1-Cys Prxs) or is involved in intermolecular (typical 2-Cys Prxs) or intramolecular (atypical 2-Cys Prxs) disulfide bridges reduced by disulfide oxidoreductases (1, 2). The involvement of a third cysteine has been very recently proposed for *Mycobacterium tuberculosis* AhpC and *Aeropyrum pernix* K1 (5, 6). According to primary sequences, Prxs can be divided into six groups: A-Prxs (2-Cys Prxs homologous to human PrxII), B-Prxs (1-Cys Prxs homologous to human PrxVI), C-Prxs (Prxs-Q), D-Prxs (type-II Prxs homologous to human PrxV), E-Prxs (bacterial thiol peroxidases), and F-Prxs (Prxs homologous to archaeal *A. pernix K1*) (2, 6–8).

The physiological electron donor for 1-Cys Prxs has been identified only recently. Although the reduction of 1-Cys B-Prxs requires glutathionylation mediated by πGST (9–10), 1-Cys D-Prxs found in plant and pathogenic bacteria were shown to catalyze a Grx/GSH-dependent reduction of hydroperoxides (11–17). Reactivation of 1-Cys B-Prx was demonstrated to occur by heterodimerization of the Prx and πGST, followed by Prx glutathionylation and the formation of an intermolecular disulfide between the Prx and πGST. The disulfide is then reduced by GSH, regenerating an active 1-Cys B-Prx (9, 10). Conversely, the mechanism of reduction of 1-Cys D-Prxs has not yet been clearly demonstrated. The sulfenic acid might be attacked by Grx reduced by GSH or might be attacked by GSH, forming a mixed disulfide bridge reduced by the Grx (12, 16, 18).

To investigate the mechanistic events that mediate the 1-Cys D-Prx regeneration, we report here a mass spectrometry and NMR study of the interaction of glutathione with 1-Cys D-Prx. This work shows that the Prx catalytic cysteine is directly glutathionylated by reaction with glutathione, and reveals that the glutathionylation of the 1-Cys D-Prx catalytic cysteine induces the dissociation of the non-covalent "perpendicular-type" homodimer into a monomer. The Prx dimerization can be reversibly achieved by the reduction of the mixed disulfide bond with dithiothreitol. These results report for the first time the existence of a dimer-monomer switch in the Prx family, similar to the decamer-dimer switch described for the 2-Cys Prxs, and give new insight into the quaternary structure modulation of the peroxiredoxins.

MATERIALS AND METHODS

Protein Samples—Samples of [U-^{15}N]Prx, C76A [U-^{15}N]Prx, and U-^{15}N, ^{13}C, 50% [^{2}H]Prx were produced as previously described (19). *Populus tremula* D-Prx NMR samples were prepared at pH 7.2 in 50 mM phosphate buffer, 10% D_2O, 0.02% NaN_3.

* This work was supported in part by the Groupement de Recherche Redoxin 2477 CNRS, France and Région Rhône-Alpes (NTAM Project 04-022-408-01 and 05-01-900-701). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental material.

[1] Recipient of a doctoral fellowship from the Ministère Délégué à la Recherche et aux Nouvelles Technologies (2003–2006).

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[3] The abbreviations used are: Prx, peroxiredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; Grx, glutaredoxin; NOE, nuclear Overhauser effect; HSQC, heteronuclear single quantum correlation; DTT, dithiothreitol.

Mass Spectrometry—Mass spectra were acquired on an API 300 triple quadrupole instrument equipped with an electrospray ion source (Sciex; Toronto, CA). For mass spectrometry-compatible non-denaturing conditions, the [^{15}N]Prx sample was submitted to seven dilution-concentration steps in a pH 7 40 mM ammonium acetate buffer using centrifugal concentrators (Nanosep). Mass spectra were acquired with a Prx sample at a concentration of 250 μM. Thereafter, GSSG was added to reach a final concentration of 2.5 mM corresponding to a 10 M excess. Mass spectra were recorded after 2 h and overnight incubation at 4 °C.

NMR Spectrometry—NMR experiments were performed at 28 °C on a Varian INOVA Unity 600 spectrometer fitted with a normal triple resonance (HCN) probe and a z-field gradient coil. ^{15}N HSQC and ^{15}N relaxation experiments were acquired with 512 complex points and a spectral width of 10000 Hz in F_2 ($^1H^N$) and 128 complex points, 2200 Hz in F_1 ($^{15}N^H$). Triple resonance experiments from the Varian Protein Pack were recorded with 512 complex points in F_3, 40 complex points in F_2, and 80 or 70 complex points for $^{13}C\alpha$, ^{13}CO, and $^{13}C\alpha\beta$ F_1 dimensions. All spectra were processed using NMRPipe scripts (20) and were analyzed with NMRView software (21).

NMR Titrations—Stock solutions of GSH and GSSG were prepared in the same buffer as the protein. Small aliquots of 10 μl of GSH or GSSG were added to a NMR tube containing 550 μl of 0.8 or 0.2 mM [^{15}N]Prx. One-dimensional ^{1}H and two-dimensional ^{1}H-^{15}N HSQC spectra were recorded at each titration point. Other conditions of glutathionylation were tested on 0.2 mM protein samples. 1-Cys D-Prx was incubated with various GSH/GSSG ratios (from 1:0.1 to 1:5) combined with oxidants (2 mM H_2O_2 or 4 mM diamide) (22). For the C76A mutant, small aliquots of 10 μl of GSSG were added to a sample containing 550 μl of 0.2 mM C76A [^{15}N]Prx up to a final concentration of 7 mM GSSG.

NMR Assignment—Triple-resonance experiments were recorded on a 0.5 mM ^{15}N, ^{13}C, 50% [^{2}H]Prx sample in the presence of 15 mM GSSG. The sequence-specific backbone resonance assignment of the protein was achieved using the following experiments: HNCO, HNCA, HN(CO)CA, and CBCA-(CO)NH. The backbone resonance assignment was confirmed with a ^{1}H-^{15}N nuclear Overhauser effect spectroscopy-heteronuclear single quantum correlation spectrum recorded with 150 ms of mixing time.

Relaxation Rate Measurements—Classical pulse sequences were used to measure longitudinal (R_1) and transverse (R_2) relaxation rates and ^{1}H-^{15}N steady-state heteronuclear NOE. NMR relaxation experiments were recorded at 28 °C on a 0.8 mM [^{15}N]Prx sample. The relaxation experiments were also performed in the presence of 8 mM at 28 °C and 15 mM GSSG at 38 °C for a comparison with previously reported data (8). For R_1 measurements of the native Prx, spectra were recorded with inversion recovery delays of 20, 200, 400, 600, 1000, 1200, and 1800 ms. Experiments were duplicated at 20, 400, and 1200 ms. For R_1 measurements in the presence of 8 or 15 mM GSSG, delays of 20, 100, 200, 400, 600, 800, 1000, and 1200 ms were used, and experiments were duplicated at 100 and 400 ms. For R_2 measurements, spectra were recorded at Carr-Purcell-Meiboom-Gill delays of 10, 30, 50, 70, 90, 110, and 130 ms, and spectra were duplicated at 50 and 110 ms. In both R_1 and R_2 experiments, the recycle delay was 4 s, and the number of transients used was 8 or 28 in the presence or not of GSSG, respectively. The heteronuclear NOE was determined from spectra recorded in the presence and absence of a ^{1}H presaturation period of 3 s within a total recycle delay of 5 s between acquisitions. The number of transients used was 140. For determination of R_1 and R_2 relaxation rates, all resonance intensities were fitted as a single exponential function of the relaxation delay in NMRView (21). The steady-state NOE values were determined in NMRView from the ratio of peak intensities obtained with and without ^{1}H saturation.

RESULTS

Prx Mass Spectrometry in the Presence of a 10 M Excess of GSSG—D-Prxs, which represent peroxiredoxins homologous to human PrxV, were previously shown to form non-covalent dimers in the reduced native state (7, 8, 23). Only D-Prxs found in Prx-Grx hybrid proteins exhibit higher molecular mass species (24). Electrospray ionization mass spectra of the 1-Cys D-Prx carried out in non-denaturing conditions (ammonium acetate buffer, pH 7) are presented in Fig. 1. As illustrated by Fig. 1*A*, the 1-Cys D-Prx is detected at a concentration of 250 μM, both as a dimeric and monomeric species. When incubated during 2 h at 4 °C with a 10 M excess of GSSG (data not shown), the peak intensities corresponding to the dimeric form strongly decrease, whereas charge states corresponding to monomeric structures of the Prx are predominant. These charge states correspond both to the native form of the Prx and to the Prx shifted by a mass increment assigned to the addition of a glutathione group. After overnight incubation with GSSG, the Prx is only detected as a monomer and predominantly as a covalent monoglutathionylated species (Fig. 1*B*). The di- and triglutathionylated species correspond to non-covalent complexes, because they are not recovered, increasing the declustering potential. These mass spectrometry results evidence that the Prx is glutathionylated in the presence of oxidized GSSG and show that the glutathionylation of the Prx induces the dissociation of the non-covalent dimer.

Prx NMR Study in the Presence of a 10 M Excess of GSSG—Interaction of GSSG with the Prx was studied by NMR ^{15}N-HSQC experiments. The ^{15}N-HSQC spectra of the free 1-Cys D-Prx and the Prx in the presence of GSSG are displayed in Fig. 2. As illustrated in Fig. 2*A*, the Prx NMR spectrum is strongly modified in the presence of a 10 M excess of GSSG (experimental conditions similar to the mass spectrometry study). The addition of 15 mM dithiothreitol (DTT) fully restores the free Prx ^{15}N-HSQC spectrum (Fig. 2*B*), showing, in agreement with the mass spectrometry results, that the NMR spectrum modifications are related to the disulfide bridge formation between the Prx and GSSG.

The longitudinal relaxation rate R_1 correlates with the overall tumbling rate τ_c of proteins, which is directly linked to their molecular weight. To characterize the R_1 perturbation with GSSG, NMR relaxation experiments were recorded with a 0.8 mM Prx sample without GSSG and in the presence of 8 mM GSSG. The addition of GSSG induces a significant increase in the average ^{15}N longitudinal relaxation rate R_1 from

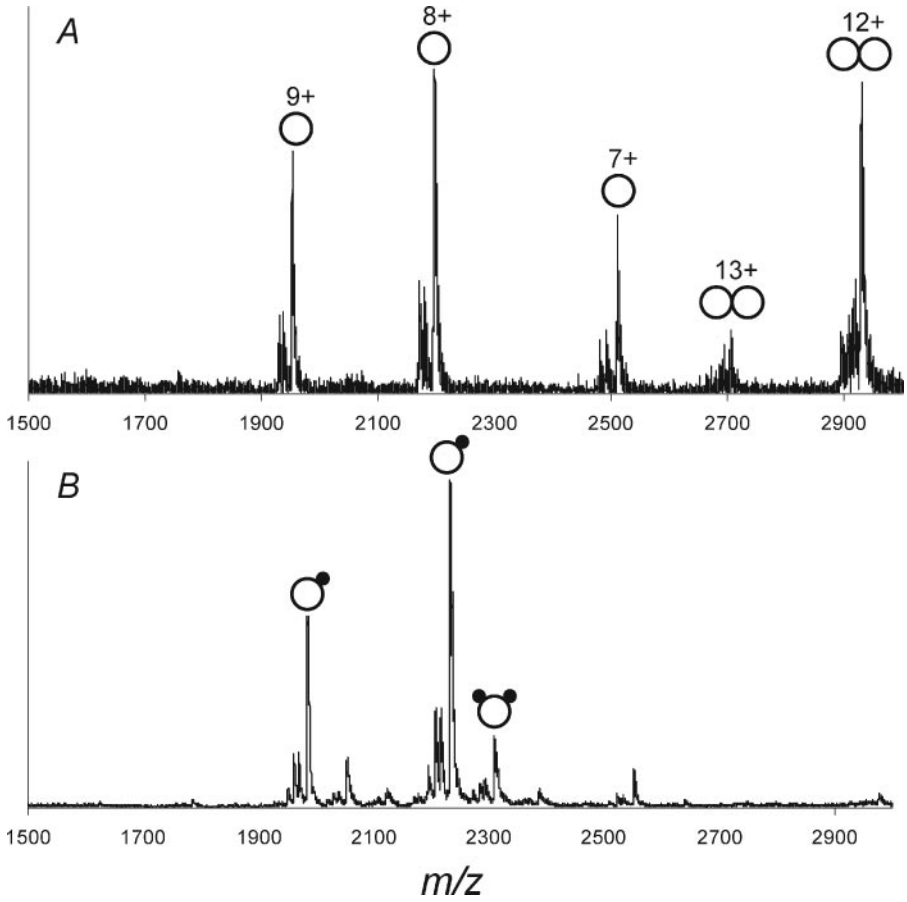

FIGURE 1. **Non-denaturing electrospray mass spectrometry of 1-Cys D-Prx (250 μM) in 40 mM ammonium acetate pH 7.** *A*, mass spectrum of the free 1-Cys D-Prx (○) results in the detection of both protein dimer and monomer in an approximative 1:1 ratio. *B*, mass spectrum of 1-Cys D-Prx mixed with 10 equivalents of GSSG (●) shows predominantly the monomeric and singly glutathionylated protein. The diglutathionylated species corresponds to a non-covalent complex.

$0.91 \pm 0.05\ s^{-1}$ to $1.72 \pm 0.19\ s^{-1}$. This clearly shows that the apparent molecular weight of the protein decreases in the presence of GSSG and fully corroborates the mass spectrometry results.

Prx Titration with GSSG—^{15}N-HSQC spectra have been recorded on a [^{15}N]Prx sample in which GSSG concentration was gradually increased from 0.1 to 20 mM. Fig. 2, *A* and *C*, show the spectrum obtained after the addition of 8 mM GSSG and 15 mM GSSG, respectively. A comparison of Fig. 2, *B* and *D*, indicates that both spectra are quite similar. However, as shown in Fig. 2, *A* and *B*, several Prx residues display a slow exchange behavior on the HSQC spectrum with 8 mM GSSG. When GSSG concentration reaches 15 mM, a single NMR signal is observed for each observable HN (Fig. 2, *C* and *D*). The addition of DTT significantly modifies the NMR spectrum giving rise to slow exchanging signals where the peaks observed in the presence of 15 mM GSSG disappear or become weaker, whereas the peaks assigned to the native reduced protein appear in the spectrum (see supplemental material).

Prx Glutathionylation in Oxidative Conditions—Protein glutathionylation occurs through different mechanisms, such as the reaction of reduced GSH with oxidized cysteine or the thiol/disulfide exchange reaction (25). Therefore, various conditions for Prx glutathionylation have been tested by incubating [^{15}N]Prx samples with different GSH/GSSG ratios (from 1:0.1 to 1:5) and oxidants such as hydrogen peroxide or diamide (22). In each case, the NMR spectra looked similar to that obtained with a 10 M excess of GSSG (see Fig. 2*A*), exhibiting slow to intermediate exchange on the NMR time scale.

Cysteine Involved in the Glutathionylation—The 1-Cys D-Prx contains two cysteine residues at positions 51 (active site cysteine) and 76 (buried cysteine located in the β4 strand). To firmly identify the cysteine involved in the disulfide bridge formation, the titration was carried out with the [^{15}N]C76A mutant. Changes similar to those of the wild-type Prx were observed in the ^{15}N-HSQC spectrum, showing that the catalytic cysteine 51, and not 76, is involved in the reaction (not shown).

Prx NMR Assignment in the Presence of 15 mM GSSG—The ^{15}N-HSQC spectrum shown in Fig. 2*C* was entirely assigned by triple resonance experiments. According to the chemical shift index calculated from the backbone chemical shifts (26) and the NOEs, no secondary structure element but the α2 helix containing the active cysteine C51 is modified in the glutathionylated Prx. The overall topology remains identical. However, the N-terminal part of the α2 helix (Thr^{50}-Val^{56}) is unwound, as evidenced from the chemical shift index, whereas HN signals in the Phe^{57}-Ala^{63} region (corresponding to α2 helix residues in the native protein) are not observed, which is likely because of a local chemical exchange excessively broadening the NMR signals (Fig. 3). The largest chemical shift differences between both Prx forms are observed at the dimer interface, as illustrated in Fig. 3: Gly^{42}–Gly^{58} (loop β3–α2 and α2 helix), Val^{79}–Asp^{103} (α3 helix and loop α3–β5), Leu^{116}–Ala^{131} (loop α4–β8), and Gly^{148}–Ser^{153} (loop β9–α5).

Prx Global Dynamic Properties in the Presence of 15 mM GSSG—The 600 MHz NMR relaxation experiments are presented in Fig. 4. For comparison, the ^{15}N R_1 and R_2 and the ^{1}H-^{15}N NOE values recorded for the native reduced protein are shown. In the presence of GSSG, the mean values for the ^{15}N R_1 and R_2 are 1.6 ± 0.05 and $11.67 \pm 0.57\ s^{-1}$, respectively. The experimental overall reorientational tumbling τ_c is 7.8 ns at 38 °C, a value in sharp contrast with the value calculated for the native dimeric protein (15.3 ± 0.9 ns at 28 °C and 14.1 ± 0.4 ns at 38 °C (8)). The experimental τ_c value of 7.8 ns is in good agreement with theoretical τ_c values calculated from the Stokes-Einstein model or from hydrodynamic calculations for a monomeric Prx structure. Moreover, the τ_c value of the Prx is

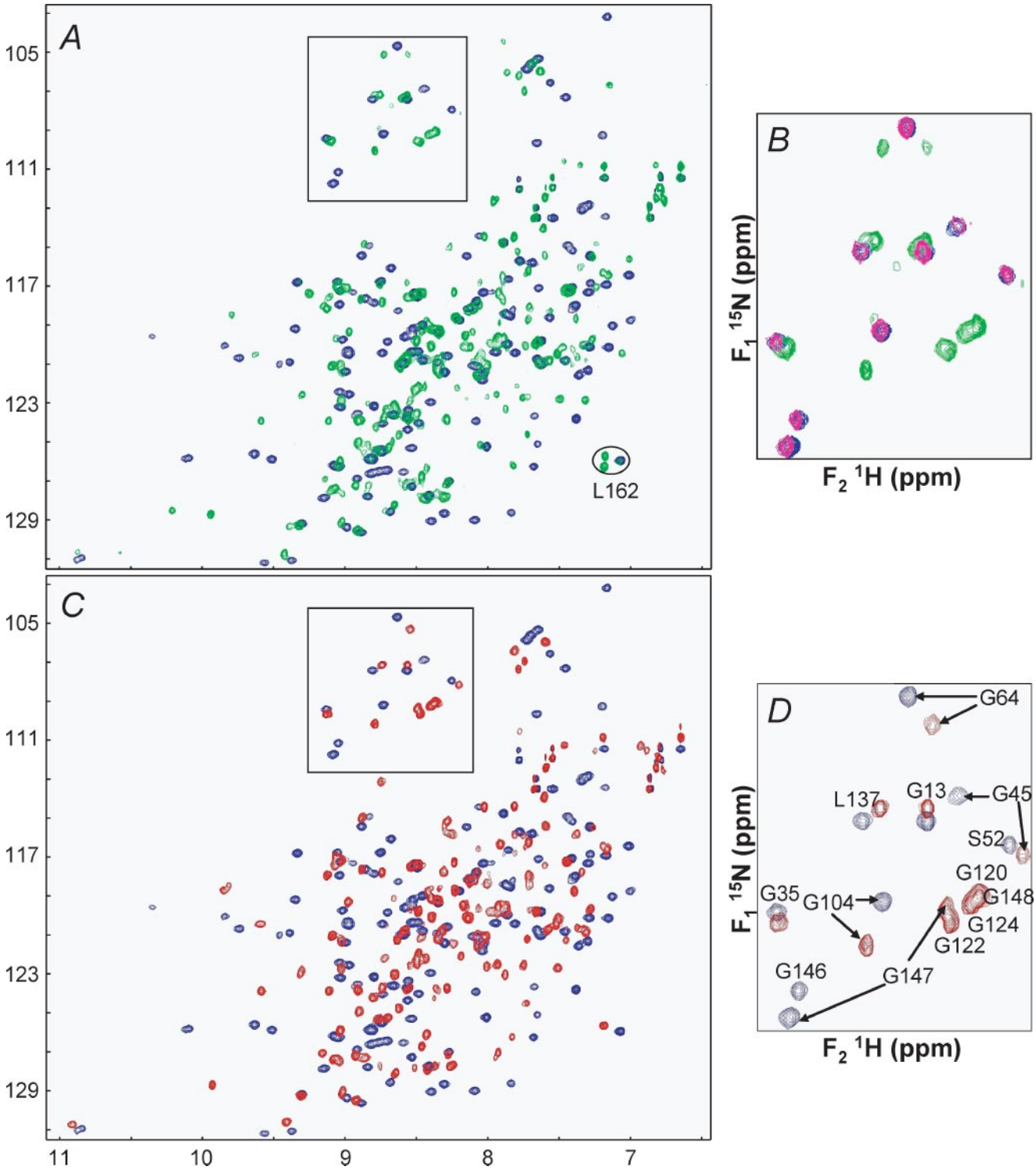

FIGURE 2. **^{15}N-HSQC NMR spectra of the 1-Cys D-Prx (0. 8 mM) recorded at 600 MHz, 28 °C.** *A*, superimposed ^{15}N-HSQC spectra of the native reduced Prx (*blue*) and the Prx in the presence of a 10 M of GSSG (8 mM GSSG, *green*). Signals of Leu^{162} are *circled* to illustrate the slow exchange behavior occurring in the presence of 8 mM GSSG. *B*, expansion of the framed spectral region shown in *A*. Shown in *magenta* is the superimposed spectrum obtained after the addition of 15 mM DTT. *C*, superimposed ^{15}N-HSQC spectra of the native reduced Prx (*blue*) and the Prx in the presence of 15 mM GSSG (*red*). *D*, expansion of the framed spectral region shown in *C*. Peak assignment is indicated for both spectra.

similar to the average experimental correlation time τ_c (7.2 ± 1.7 ns) measured on 9 proteins with molecular weights similar to that of the monomeric Prx (17.9 ± 1.4 kDa) (27). The relaxation experiments therefore clearly evidence that the protein is monomeric in the presence of 15 mM GSSG. As shown in Fig. 2, the reducing agent DTT does fully restore the dimeric Prx form when the protein is incubated with 8 mM GSSG, whereas a small portion of protein remains in the monomeric state after incubation with DTT in the presence of 15 mM GSSG (see supplemental material). This indicates that the spectrum

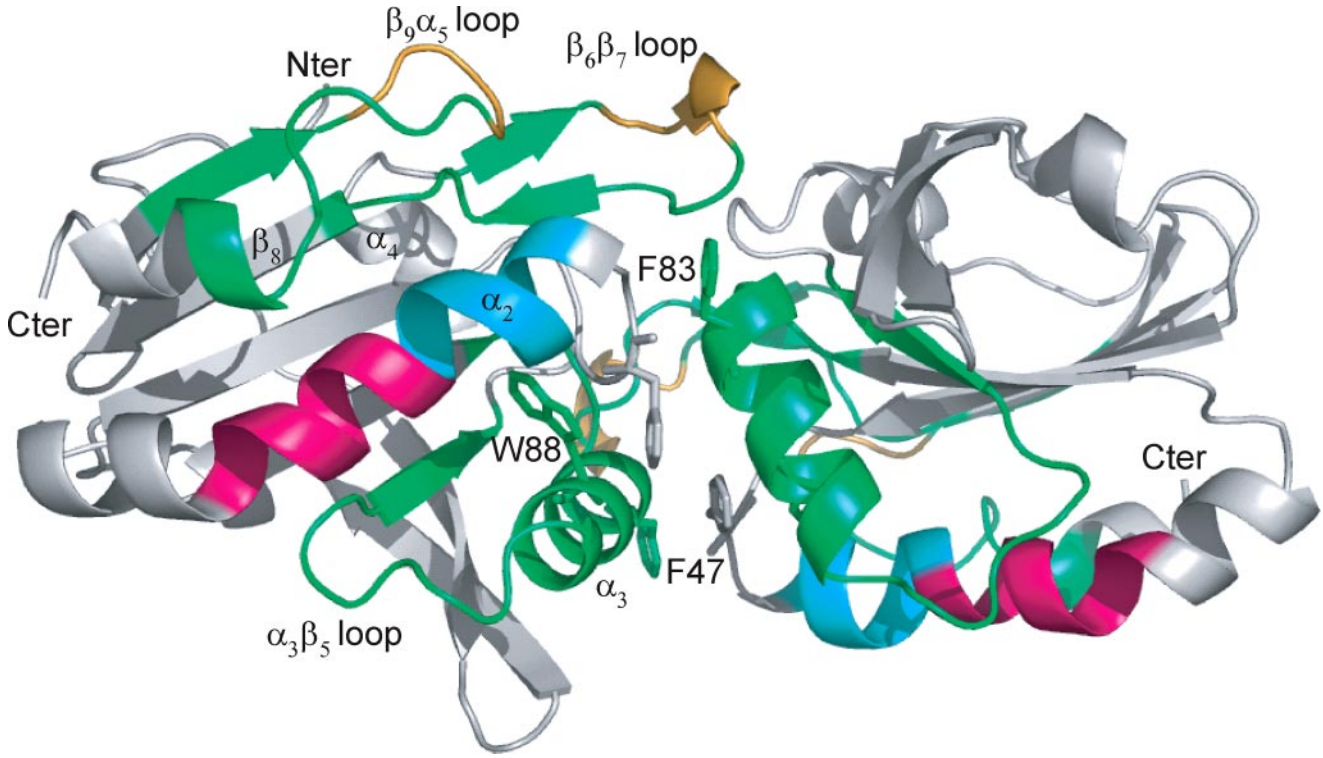

FIGURE 3. **Three-dimensional structure of the 1-Cys D-Prx dimer (Protein Data Bank entry 1TP9) illustrating the Prx-Prx interface perpendicular to the central β-sheet.** The peptidic regions exhibiting a different behavior in the NMR experiments without GSSG or in the presence of 15 mM GSSG are shaded as follows: *cyan*, the α2 helix region (Thr^{50}–Val^{56}) that unwinds in the presence of GSSG; *pink*, the Pro^{57}–Ala^{63} region for which the HSQC signals are not observed with GSSG; *green*, the regions where large chemical shift differences are observed in the presence of GSSG, the Gly^{42}–Gly^{58} (loop β3–α2 and α2 helix), Val^{79}–Asp^{103} (α3 helix and loop α3–β5), Leu^{116}–Ala^{131} (loop α4–β8), and Gly^{148}–Ser^{153} (loop β9–α5) regions; *orange*, the β6–β7 (Gln^{119}–Gly^{122}) and β9–α5 (Gly^{146}–Glu^{149}) loops that become very flexible in the monomeric state (see Fig. 4).

modifications displayed in Fig. 2*C* are not exclusively related to the disulfide bridge formation between Prx and GSSG and suggests that the GSSG molecule facilitates the dimer dissociation through non-covalent binding to the Prx, a process not reversed by DTT.

As illustrated in Figs. 3 and 4, local dynamic modifications in the picosecond–nanosecond time scale are also observed in the monomeric Prx. The relaxation experiments indicate that β6–β7 (Gln^{119}–Gly^{122}) and β9–α5 (Gly^{146}–Glu^{149}), loops both containing three glycine residues, are very flexible in the monomeric state, whereas those loops are structurally constrained in the dimeric protein (Figs. 3 and 4).

DISCUSSION

Under moderate oxidative stress, cysteine residues can undergo reversible oxidation by forming mixed disulfides with protein thiol groups or with low molecular mass thiols such as glutathione, the concentration of which reaches millimolar concentrations in cells (25, 28, 29). Such a modification is thought to play a protective role against irreversible oxidation or to modulate protein function (25, 28, 29). The Prx glutathionylation could prevent the formation of sulfinic or sulfonic acids, although typical 2-Cys Prx sulfinic acids can be reduced by sulfiredoxins (30–32). The glutathionylation of Prxs has been observed under oxidative stress conditions in different proteomic studies (33–35) and has been recently reported as an intermediate step for 1-Cys B-Prx regeneration (9, 10). The reduction of 1-Cys B-Prxs (Prxs homologous to human PrxVI) was shown to require glutathionylation mediated by πGST through the formation of a heterodimer between Prx and πGST (9, 10). A different reduction system is used by 1-Cys D-Prxs found in plant and pathogenic bacteria (Prxs homologous to human PrxV), which catalyze a Grx/GSH-dependent reduction of hydroperoxides (11–18). The mechanism of 1-Cys D-Prx regeneration is more hypothetical (16, 18).

The mass spectrometry and NMR studies presented here show that the catalytic cysteine of the 1-Cys D-Prx can react directly with glutathione, in agreement with the observation that the recombinant 1-Cys D-Prx from *Haemophilus influenzae* was purified as a glutathionylated protein (16). The slow exchange behavior observed for several residues on the NMR spectra indicate that the Prx glutathionylation is not complete *in vitro* (see supplemental material). However, the Prx glutathionylation *in vivo* cannot be anticipated. From the mass spectrometry and NMR results, a mechanism for the 1-Cys D-Prx regeneration can be proposed (Fig. 5). In this mechanism, the reduction of the D-Prx sulfenic acid involves the intermediate formation of a mixed disulfide with glutathione, reduced in turn by the Grx (Fig. 5). In this case, the Grx interacts with a Prx monomeric glutathionylated form and plays a role similar to that of πGST for 1-Cys B-Prx reduction. However, the marked difference between 1-Cys B-Prxs and 1-Cys D-Prxs is their glutathionylation mechanism, because B-Prxs do not react directly with glutathione.

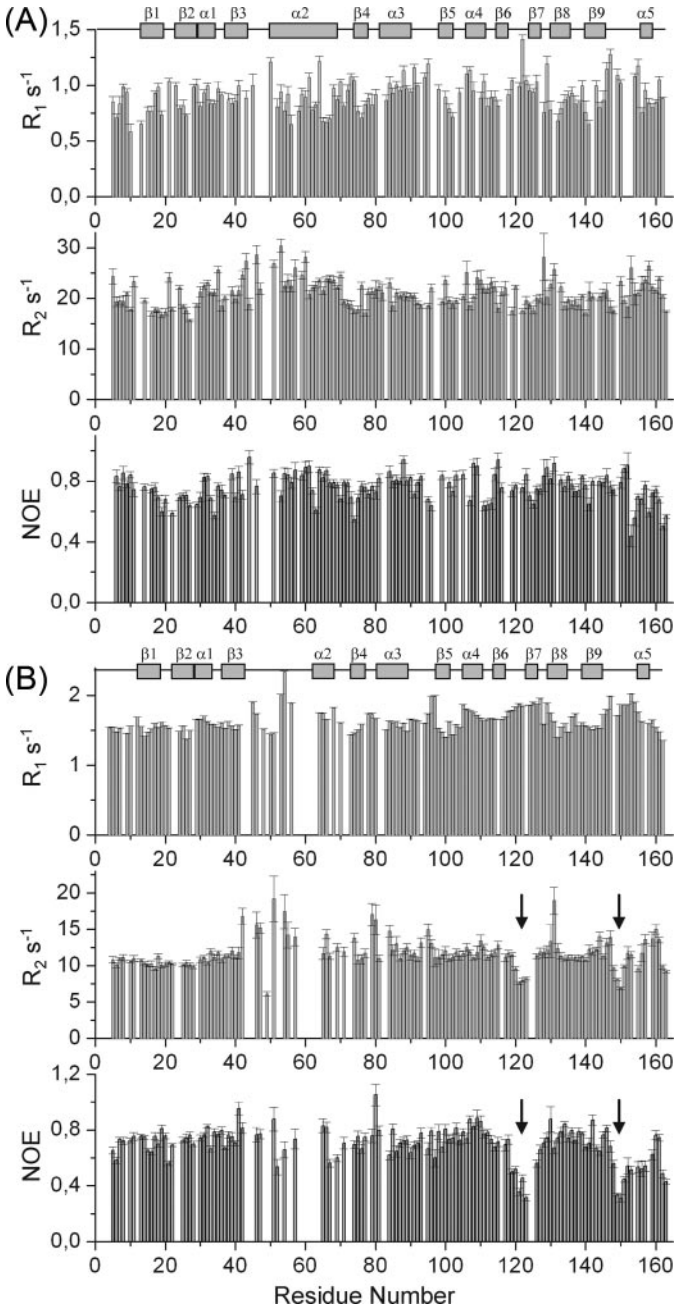

FIGURE 4. **Prx ^{15}N-relaxation parameters R_1, R_2, and ^{1}H-^{15}N NOE as a function of the protein sequence.** The *error bars* represent standard deviations. *A*, relaxation parameters of the native reduced Prx without GSSG. *B*, relaxation parameters of the Prx in the presence of 15 mM GSSG. *Arrows* indicate the $\beta6-\beta7$ (Gln^{119}–Gly^{122}) and $\beta9-\alpha5$ (Gly^{146}–Glu^{149}) loops, for which a marked NOE and R_2 value decrease is observed.

The mass spectrometry and NMR studies presented here reveal that the glutathionylation of the 1-Cys D-Prx catalytic cysteine induces the dissociation of the so-called perpendicular-type dimer into monomer. In addition, as illustrated in Fig. 3, NMR experiments indicate that the Prx dimer dissociation is combined with structural modifications (such as the $\alpha2$ helix unwinding) and dynamic changes ($\beta6-\beta7$ and $\beta9-\alpha5$ loops). This demonstrates that a new Prx isoform is obtained when the protein is glutathionylated.

All Prxs share a common fold which consists of a central seven-strand β-sheet surrounded by five helices. Structural

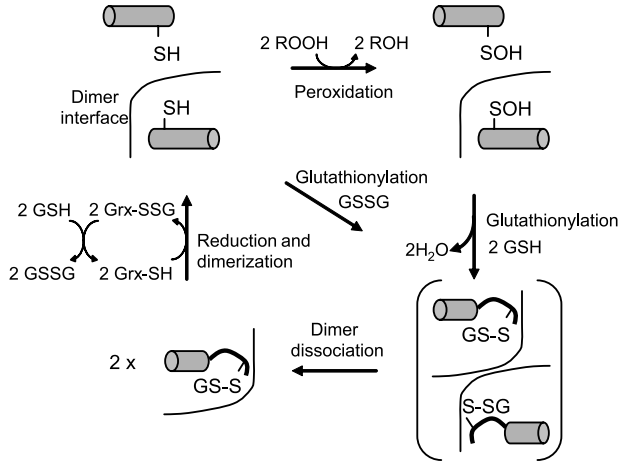

FIGURE 5. **Proposed mechanism for the 1-Cys D-Prx catalytic cycle.** The 1-Cys D-Prx is regenerated with the glutathione-glutaredoxin system. The glutathionylation of the catalytic cysteine residue induces the unwinding of the $\alpha2$ helix and the destabilization of the dimer, which dissociates. The Prx is regenerated by reaction with Grx.

studies have revealed that the Prxs can form two types of homodimers, with a Prx-Prx interface either parallel or roughly perpendicular to the central β-sheet (reviewed in Refs. 8 and 23). The "parallel" association is observed in A-Prx, B-Prx, and F-Prx dimers, whereas the perpendicular association is found in D-Prx dimers, E-Prx dimers, and in A-Prx and F-Prx toric decamers or dodecamers. Very recently, octamers were observed in *M. tuberculosis* AhpE crystals (36), whereas C-Prxs were characterized as monomers (37).

The redox-dependent stability of the perpendicular interface differs between the Prx groups. In 2-Cys A-Prxs, the intermolecular disulfide bridge formation destabilizes the decamer, which dissociates into oxidized dimers (38–41). The perpendicular interface breaking is triggered by the unwinding of the $\alpha2$ helix and is followed by structural rearrangements of loops located at the interface (38–41). In particular, changes in the rotamers of aromatic residues from the $\beta3-\alpha2$ loop (Phe^{48} in rat PrxI) and $\alpha3$ helix (Phe^{82} and Trp^{87}) have been observed (38–41). Similarly, the N-terminal part of the 1-Cys D-Prx $\alpha2$ helix is unwound, as shown by NMR experiments. Moreover, the three aromatic residues are conserved in 1-Cys D-Prxs (Fig. 3), suggesting that the interface breaking of the 1-Cys D-Prx involves structural events similar to those observed for 2-Cys Prxs. In contrast, the formation of an intramolecular disulfide bridge does not induce the dissociation of the E-Prx (EcTPx) dimer, the stability of which is allocated to the replacement of Phe^{82} by an aspartate residue involved in electrostatic interactions with an arginine residue of the second monomer (41).

The work reported here shows that the D-Prxs are not obligate dimers, which correlates with the small surface area (<800 Å^2) of their Prx-Prx perpendicular interfaces (8, 42). As previously postulated, the Prx dimerization through the perpendicular interface could serve to shape the active site in the reduced active protein and consequently modulate the specificity of the Prx toward the peroxide substrate (4, 41, 43).

The redox-dependent modulation of the 1-Cys D-Prx quaternary structure between dimer and monomer reported here is

similar to the decamer-dimer switch described for 2-Cys Prxs. In both cases, the perpendicular interface dissociates when the Prx is oxidized through the formation of a disulfide bond involving the catalytic cysteine. Although the glutathionylation of Prxs under oxidation stress has been reported in many proteomic studies (33–35), the breaking of the perpendicular interface by glutathionylation is reported for the first time in the Prx family. To understand why some Prxs can react directly with glutathione while other Prxs cannot, experiments will be carried out to solve the three-dimensional structure of the glutathionylated Prx and reveal the GSH binding site.

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