How pH Modulates the Dimer-Decamer Interconversion of 2-Cys Peroxiredoxins from the Prx1 Subfamily*

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Background: Oligomeric changes affect the function of typical 2-Cys peroxiredoxins.

Results: pH decrease favors decamerization and this effect depends on His113 and Asp76.

Conclusion: Protonated His113 attracts Asp76 inducing a conformational change that stabilizes the decamer.

Significance: Learning how pH modulates the oligomerization of typical 2-Cys peroxiredoxins is an important step into understanding the pH effect on their function.

2-Cys peroxiredoxins belonging to the Prx1 subfamily are Cys-based peroxidases that control the intracellular levels of H2O2 and seem to assume a chaperone function under oxidative stress conditions. The regulation of their peroxidase activity as well as the observed functional switch from peroxidase to chaperone involves changes in their quaternary structure. Multiple factors can modulate the oligomeric transitions of 2-Cys peroxiredoxins such as redox state, post-translational modifications, and pH. However, the molecular basis for the pH influence on the oligomeric state of these enzymes is still elusive. Herein, we solved the crystal structure of a typical 2-Cys peroxiredoxin from Leishmania in the dimeric (pH 8.5) and decameric (pH 4.4) forms, showing that conformational changes in the catalytic loop are associated with the pH-induced decamerization. Mutagenesis and biochemical studies revealed that a highly conserved histidine (His113) functions as a pH sensor that, at acidic conditions, becomes protonated and forms an electrostatic pair with Asp76 from the catalytic loop, triggering the decamerization. In these 2-Cys peroxiredoxins, decamer formation is important for the catalytic efficiency and has been associated with an enhanced sensitivity to oxidative inactivation by overoxidation of the peroxidatic cysteine. In eukaryotic cells, exposure to high levels of H2O2 can trigger intracellular pH variations, suggesting that pH changes might act cooperatively with H2O2 and other oligomerization-modulator factors to regulate the structure and function of typical 2-Cys peroxiredoxins in response to oxidative stress.

Peroxiredoxins (Prxs) are key components for antioxidant protection and cell signaling pathways. They are classified into distinct subfamilies, which are active site architectures and sequence similarity. The Prx1 subfamily comprises typical 2-Cys Prxs that are highly expressed and found in all living kingdoms. In eukaryotes, Prx1 subfamily members are present not only in the cytosol but also in organelles such as mitochondrion, chloroplast, and endoplasmic reticulum. This subfamily includes the bacterial AhpC proteins, the human Prxs I to IV, the yeast thiol-specific antioxidant protein, and the tryparedoxin peroxidases.

Prx1 subfamily members are obligate homodimers that display two identical active sites located in opposite sides of the dimer interface. In most cases, Prx1 homodimers can associate noncovalently forming doughnut-like assemblies that are usually decameric. The dimer-decamer interconversion seems to play a role during the catalytic cycle, influencing the catalytic efficiency. Some studies support that Prx1 subfamily members can further form higher-order oligomers, which seems to switch their function from peroxidase to chaperone.

The redox state of the peroxidatic cysteine (Cp) is the best characterized factor regulating the oligomerization of Prx1 subfamily members. When Cp is reduced, the catalytic loop and C-terminal extension adopt a fully folded (FF) conformation, which stabilizes the decameric form. By reducing peroxides (ROOH) to ROH, Cp is oxidized to sulfenic acid (Cp-SOH) and the active site becomes locally unfolded (LU), allowing the formation of a disulfide bond between Cys-SOH and the resolving cysteine (Cr), located in the other subunit forming the catalytic interface. This conformational change favors the dissociation of decam-
ers into dimers (10, 13). Under oxidative stress, some Prx1 subfamily members can be inactivated by C_p, overoxidation and assume higher-order oligomeric states that display chaperone function as shown in vitro (11, 12, 14).

Besides the redox state, other factors such as post-translational modifications, ionic strength, and pH can also affect the equilibrium of Prx1 oligomers (15). For the human Prx I, phosphorylation of Thr^{90} triggers the formation of high molecular weight complexes and seems to induce the functional switch to chaperone (16), whereas Cys^{83} glutathionylation (17) and Cys^{72} nitrosylation (18) favor decamer dissociation. The ionic strength seems to have opposing effects depending on the Prx1 subfamily member. High ionic strength (>0.3 M NaCl) stabilizes the decameric form of Prx1 members from plant and bacteria, but favors the dimeric form of the rat Prx I (7, 19, 20). Acidification favors the decameric state of plant and mammalian Prx1 subfamily members (7, 21). The dimer-decamer equilibrium of these enzymes is highly responsive to small pH variations in the physiological range (between pH 7 and 8), suggesting that a dissociable group with a pK_a value in this pH interval affects the equilibrium between the two oligomeric states (7, 21).

Intracellular pH variations play an important and underappreciated role in physiological and pathological situations such as apoptosis, neurotransmission, and insulin secretion in mammals as well as CO_2 fixation in plants (22, 23). It has been shown that such pH variations can modulate the structure and function of proteins, including caspasess (22, 24) and enzymes belonging to the Prx1 subfamily (7). Nevertheless, the molecular mechanisms by which pH controls Prx1 oligomerization remain elusive.

Therefore, we investigated the structural basis for the pH-dependent decamerization of Prx1 subfamily members using as a model the mitochondrial tryptaredox peroxidase from Leishmania braziliensis, here named LbPrx1m according to the nomenclature proposed by Gretes et al. (25). Up-regulation of this protein has been associated with protection against apoptosis-like death (26) and resistance to miltefosine (27), a drug whose activity is linked to apoptosis and disturbance of lipid- metabolism (28) and resistance to miltefosine (27). Moreover, Leishmania Prx1m confers thermotolerance to the parasite and displays in vitro chaperone activity, which seems to be crucial for virulence (29). In this work, we solved the crystal structure of LbPrx1m at basic and acidic conditions, providing, for the first time to our knowledge, structural data of a Prx1 subfamily member in both dimeric and decameric states induced by pH variations. Structural comparisons along with site-directed mutagenesis and biophysical studies revealed that His^{113} functions as a pH sensor. With decreasing pH, His^{113} becomes protonated and attracts Asp^{76}, inducing a conformational change in the catalytic loop that promotes the decamerization. The ionic pair, His^{113} and Asp^{76}, is present in most Prx1 subfamily members, suggesting that this mechanism is conserved in typical 2-Cys Prxs.

**EXPERIMENTAL PROCEDURES**

Molecular Cloning and Site-directed Mutagenesis—The LbPrx1m gene (RefSeq XM_001562186.1) was cloned into a pET28a-His-TEV vector between NdeI and Sall restriction sites as described in Ref. 30. The Asp^{76} → Ala and His^{113} → Ala mutants were produced using the QuikChange™ site-directed mutagenesis kit (Stratagene).

**Protein Expression and Purification**—The wild-type (wt) LbPrx1m and mutants were expressed in Escherichia coli BL21(DE3)ASlyD cells harboring plasmid pRARE2 as described (30). For protein purification, cells were resuspended in buffer A (20 mM sodium phosphate, pH 7.4, 500 mM NaCl, and 5 mM imidazole) containing 2 mM PMSF, disrupted by sonication and centrifuged at 20,000 × g for 30 min (277 K). The supernatant was loaded onto a nickel-charged Hi-trap chelating column (5 ml, GE Healthcare) coupled to an Akta FPLC system (GE Healthcare). After washing the resin with 10 column volumes of buffer A, bound proteins were eluted using a non-linear imidazole gradient from 5 to 1000 mM. Fractions containing the target protein were dialyzed against 25 mM Tris-HCl (pH 7.5) and loaded onto a Q-Sepharose Fast Flow column (5 ml, GE Healthcare). The target protein was eluted with 0.15 M NaCl during a 0–1 M NaCl gradient. The fractions were pooled, concentrated, and subjected to a size-exclusion chromatography using a HiLoad 16/600 Superdex 200 column (GE Healthcare) pre-equilibrated with 25 mM Tris-HCl (pH 7.5). The protein was purified and analyzed under non-reducing conditions, unless otherwise stated. The oxidation state of samples was verified by SDS-PAGE under reducing and non-reducing conditions.

Analytical Size Exclusion Chromatography (aSEC)—Analytical SEC experiments at different pH values were carried out on a Superdex 200 10/300 GL column (GE Healthcare) using a flow rate of 0.5 ml min^{-1}. LbPrx1m samples, previously incubated with the Chelex\(^{10}\) 100 resin (Bio-Rad), were diluted to 48 μM using the respective aSEC buffers and loaded onto the column pre-equilibrated with 50 mM MMT (DL-malic acid, MES, and Tris base), pH values of 4, 5, 6.7, or 8, 150 mM NaCl, and 10 mM EDTA. The D76A and H113A mutants (48 μM), previously incubated at pH 4.0 (50 mM MMT, pH 4, 150 mM NaCl, 10 mM EDTA) or at pH 7.0 with DTT (25 mM Tris-HCl, pH 7.0, 150 mM NaCl, 5 mM EDTA, and 2 mM DTT) by 30 min, were loaded onto the column pre-equilibrated with the corresponding buffers. To evaluate whether the N-terminal His tag and the protein redox state influence the pH effect on the dimer-to-decamer conversion, the same assay was performed with LbPrx1m pre-treated with TEV protease. For the cleavage, LbPrx1m at 1 mg ml^{-1} was treated with His-tagged TEV protease (0.01 mg ml^{-1}) for 30 min at 298 K. The samples were loaded onto a nickel-nitrilotriacetic acid Superflow resin (Qiagen) to separate the cleaved protein (collected from the flow-through) from the remaining uncleaved LbPrx1m and His-tagged TEV protease. Analytical SEC runs were performed in the presence and absence of 2 mM DTT, in 25 mM Tris-HCl (pH 7.0, 7.5, and 8.0) containing 150 mM NaCl and 5 mM EDTA. All these experiments were carried out under chelating conditions because some divalent cations stabilize the LbPrx1m decamer at alkaline pH values. The SEC column was calibrated using low and high molecular weight markers (GE Healthcare).

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The pH-induced Decamerization of Prx1 Subfamily Members

Quantification of Free Thiol Groups—LbPrx1m samples (48 μM) in 25 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 5 mM EDTA were treated with 2 mM DTT for 30, 60, 120, and 240 min at room temperature. Excess DTT was removed using the HiTrap Desalting column (5 ml, GE Healthcare). The samples were incubated with 100 μM DTNB for 1 h. The 2-nitro-5-thiobenzoate production was monitored spectrophotometrically at 412 nm (ε_{412} = 14,150 M⁻¹ cm⁻¹) and the eluted protein concentrations were used to calculate the number of free thiols per molecule.

Dynamic Light Scattering (DLS)—DLS measurements were performed under non-reducing conditions on a Dynapro Molecular Sizing instrument at 291 K. Protein samples at 48 μM were previously centrifuged for 20 min at 20,000 × g. Data were collected with intervals of 10 s with at least 100 acquisitions. The diffusion coefficient (D) was determined from the analysis of measured time-dependent fluctuations in the scattering intensity and used to calculate the hydrodynamic radius (R_{H}) of the protein via Stokes-Einstein equation. Data analysis was performed using the software Dynamics V6.3.40.

Crystallization, Data Collection, Processing, and Structure Determination—LbPrx1m sample was concentrated to 10 mg ml⁻¹ for crystallization trials. Crystals of the dimeric form were grown by the sitting-drop vapor-diffusion method in drops containing 0.5 μl of the protein sample mixed with an equal volume of reservoir solution (27% (w/v) PEG4000, 0.1 M Tris-HCl, pH 8.5, 0.2 M Li₂SO₄) added by 5 mM EDTA at 291 K. The decameric form was crystallized in 0.1 M MMT buffer (pH 4.4), HCl, pH 8.5, 0.2 M Li₂SO₄) added by 5 mM EDTA at 291 K. The pH-induced Decamerization of Prx1 Subfamily Members

RESULTS

Acidification Shifts the Dimer-Decamer Equilibrium to the Decameric State—LbPrx1m purified under non-reducing conditions assumes the disulfide-bonded state (SS), as shown by SDS-PAGE analysis (Fig. 1A). Analytical SEC experiments of this sample at different pH values revealed that decreasing pH induces the oxidized LbPrx1m dimers to associate into decamers (Fig. 1C). At pH 8, LbPrx1m was essentially dimeric, whereas at pH ≤ 7 LbPrx1m was predominantly decameric, indicating that the decamer-to-dimer transition occurs in a narrow pH range between pH 7 and 8.

Corroborating the aSEC results, DLS measurements of oxidized LbPrx1m (48 μM) at pH 8 and 4 indicate averaged R_{H} of 2.9 and 5.7 nm, respectively (Fig. 1D). These values are in agreement with the theoretical R_{H} estimated for the dimer (3.1 nm) and decamer (6.1 nm) based on the molecular mass of the respective oligomers (46 and 230 kDa).

To evaluate the influence of the protein redox state on this transition, we analyzed samples treated with 2 mM DTT (Fig. 1F). Under this condition, LbPrx1m assumes a reduced state, according to SDS-PAGE analysis and thiol quantification (Fig. 1, A and B). The DTT-treated samples maintained a number of free thiols close to three, which is consistent with the number of cysteines of LbPrx1m (Cys₁ⁿ, Cys₁⁰ᵗ, and Cys²⁰₄). Analytical SEC experiments with or without DTT were performed at different pH conditions using TEV-cleaved LbPrx1m (without His tag) (Fig. 1, E and F). Unlike the mitochondrial Prx III (41), removal of the His tag did not influence the dimer-decamer equilibrium of oxidized LbPrx1m. Regarding the reduced protein, it remained dimeric at pH 8 and decameric at pH 7, similarly to the oxidized form (Fig. 1, E and F). However, treatment with DTT increased the decameric fraction at pH 7, suggesting that, at this pH, reduced decamers are more stable than those oxidized. At pH 7.5, the oxidized protein remained predominantly dimeric, whereas those that were reduced eluted as a mixed population of dimers, intermediate species, and decamers. These results indicate that, at this pH, Cₚ reductase is not sufficient to fully convert dimers into decamers.

TABLE 1

| Data collection and refinement statistics | Decamer | Dimer |
|-----------------------------------------|---------|-------|
| Space group | P2₁,2,2₁ | P4₁,2,2 |
| Cell dimensions | a, b, c (Å) | 90.2, 98.9, 228.1 | 132.5, 132.5, 44.6 |
| Molecules per AU | 10 | 2 |
| Resolution (Å) | 40.0-2.9 (3.1-2.9) | 40.0-2.8 (3.0-2.8) |
| Unique reflections | 397,953 (54,334) | 137,931 (18,831) |
| R_{merge} (%) | 25.4 (219.5) | 20.0 (322.1) |
| R_{free} (%) | 26.9 (234.8) | 20.9 (335.5) |
| I/σ(I) | 9.9 (0.9) | 10.6 (0.9) |
| CC_{1/2} | 1.0 (0.5) | 1.0 (0.5) |
| Completeness (%) | 99.5 (97.6) | 98.7 (92.9) |
| Multiplicity | 9 (8) | 13 (13) |

*AU, asymmetric unit.

Values in parentheses are for highest-resolution shell.

CC_{1/2}, correlation between intensities from random half-datasets (51).
Thus, collectively, these data point out that \textit{LbPrx1m} dimer-decamer equilibrium is susceptible to small variations around physiological pH, being shifted to the decameric state upon acidification and to the dimeric state at mild basic conditions, regardless of whether \textit{C_{Pr}} is reduced or disulfide bonded.

\textit{LbPrx1m} crystallized as a dimer at basic pH and as a decamer at acidic pH—In accordance with aSEC and DLS results, \textit{LbPrx1m} crystals grown at pH 8.5 presented a dimer in the asymmetric unit, whereas those grown at pH 4.4 displayed a decamer. Energetic analyses using PDBePISA (42) confirmed that the dimer found in the asymmetric unit is the most stable assembly detected in the crystal, indicating the absence of higher order oligomers generated by symmetry.

The \textit{LbPrx1m} decamer is a doughnut-like structure formed by the association of five homodimers (Fig. 2). Each homodimer is stabilized by an anti-parallel intermolecular association between \textit{\beta} strands from both subunits (Fig. 2A). This interface is named by Sarma and colleagues (43) as B-type interface (B for “\textit{\beta} sheet” based). To form the decamer, five B-type dimers interact each other via dimer-dimer interfaces named A-type (A for alternate or ancestral) (43) (Fig. 2B).

\textit{LbPrx1m} is oxidized in both crystal structures. In the dimeric crystalline form, the electronic density map allowed the modeling of the disulfide bond between \textit{Cys}_{81} (\textit{C_{Pr}}) from chain B and \textit{Cys}_{204} (\textit{C_{R}}) from chain A (Fig. 2, A and C). In the other active site, part of the catalytic loop (\textit{C_{Pr}}-loop) and the whole C-terminal extension (after residue Asn198) are disordered, probably due to a higher flexibility of this region. In all decamer subunits, the \textit{C_{Pr}}-loop is well ordered and assumes a locally unfolded conformation, exposing \textit{C_{Pr}}. The electron density map allowed the modeling of the disulfide bond of \textit{C_{Pr}} from subunits A, C, and G with \textit{C_{R}} from subunits B, D, and H, respectively (Fig. 2, B and D). In the remaining chains, the region from Asn_{198} to Asn_{235} is disordered. A similar pattern was already observed in the oxidized forms of Prx IV (44) and AcePrx-1 (45) and can be either due to inherent flexibility of \textit{C_{R}} and adjacent residues or due to disulfide bond disruption by radiation damage during data collection.

\textit{His}^{113} is a pH Sensor for Decamerization—Comparison between the dimeric (pH 8.5) and decameric (pH 4.4) structures of \textit{LbPrx1m} showed that the \textit{C_{Pr}}-loop is locally unfolded and undergoes conformational changes upon decamerization.

**FIGURE 1.** Acidification shifts the dimer-decamer equilibrium to the decameric state. A, SDS-PAGE under non-reducing conditions of \textit{LbPrx1m} samples (3 \textmu g) showing reduced monomers (SH) upon pre-treatment with 2 mM DTT (+) and disulfide-bonded (SS) dimers in the absence of DTT (−). B, content of \textit{LbPrx1m} thiols determined by a DTNB assay with samples pre-treated with 2 mM DTT for 30, 60, 120, and 240 min. The values correspond to the calculated average of three independent experiments. C, SEC chromatograms of oxidized (SS) \textit{LbPrx1m} (wt) at 48 \mu M. The protein was pretreated with Chelex 100 (Bio-Rad) and loaded onto a column pre-equilibrated with 50 mM MMT (pH 4–8), 10 mM EDTA, and 150 mM NaCl. Numbers above the graphic represent the molecular masses (kDa) of standard proteins used for column calibration. Gray dotted lines indicate their respective elution volumes. D, DLS assays of oxidized \textit{LbPrx1m} (wt) at 48 \mu M in pH 4 and 8. R_{h} = hydrodynamic radius (nm), %Pd = polydispersity (%), %M = fraction of mass (%) corresponding to the peak. E and F, SEC chromatograms of TEV-cleaved \textit{LbPrx1m} at 48 \mu M under non-reducing conditions (E) and in the presence of 2 mM DTT (F) in 25 mM Tris-HCl buffer (pH 7.0, 7.5, and 8.0) containing 5 mM EDTA and 150 mM NaCl.
In the dimeric form, this loop assumes an open conformation that exposes to the surface the residues Asp76, Asp108, and Ser112 (Fig. 3A). Conversely, in the decameric form, an intramolecular hydrogen bond network involving Tyr73, Asp76, Ser106, Asp108, and Ser112, as well as electrostatic interactions of His113 with Asp76, locks the CP-loop in a closed conformation (Fig. 3B). Upon this conformational change, Phe77 and Phe79 are placed at orientations that permit hydrophobic contacts with residues Ile55, Tyr111, Leu114, and Ala115 from the adjacent subunit, favoring the decameric assembly (Fig. 3B).

Among these residues, only His113 might have its protonation state affected in the pH interval where the dimer-to-decamer transition was observed. Changes in the His113 side chain protonation probably contribute with decamer stabilization through electrostatic interactions with Asp76 at pH below 8.0.

To test the hypothesis that His113 is relevant for the pH-induced decamerization of LbPrx1m and evaluate the role of Asp76 in this process, we performed aSEC experiments with H113A and D76A mutants in the presence (pH 7) and absence (pH 4) of reducing agent (Fig. 3C). Samples treated with DTT were analyzed in neutral pH because the reducing power of DTT is lowered at acidic medium.

As expected, the H113A mutant eluted as a dimer in both conditions, demonstrating that LbPrx1m depends on His113 to decamelize in neutral to acidic conditions regardless of whether Cp is reduced or disulfide bonded (Fig. 3C). The D76A mutant also eluted as a dimer when reduced and displayed two elution peaks when oxidized. DLS analysis of this sample indicated an average $R_H$ of 3.5 nm and a polydispersity of 27%, supporting the predominance of D76A dimers observed in the aSEC profile (Fig. 3C and D). Thus, aSEC and DLS data show that D76A substitution prevented the pH-induced decamerization of LbPrx1m independent of the Cp redox state.

Together, these results indicate that the ionic interaction involving His113 and Asp76 side chains is pH-sensitive and crucial for decamerization upon acidification of LbPrx1m in both reduced and disulfide-bonded states. Supporting this conclusion, the theoretical $pK_a$ of His113 and Asp76 side chains in the decamer structure, calculated using the program PROPKA 3.0 (46), are 7.37 and 2.87, respectively, indicating that pH variations in the 7.0 to 8.0 interval might affect the protonation state of His113 and consequently the ionic interaction with the negatively charged Asp76, which correlates with the dimer-to-decamer transition occurring in this pH range (Fig. 1C).

**DISCUSSION**

This work reveals that acidification in a narrow and physiological pH range favors the decamerization of both oxidized and reduced forms of LbPrx1m, a typical 2-Cys Prx from protozoa. The same phenomenon has already been observed for oxidized forms of typical 2-Cys Prxs from plant and mammal (7, 21), but the structural basis for this pH effect was unknown so far.

Based on crystallographic, site-directed mutagenesis, and biophysical studies, we proposed a model to explain how pH modulates the dimer-decamer equilibrium of LbPrx1m (Fig. 2A and B). In the dimeric form, this loop assumes an open conformation that exposes to the surface the residues Asp76, Asp108, and Ser112 (Fig. 3A). Conversely, in the decameric form, an intramolecular hydrogen bond network involving Tyr73, Asp76, Ser106, Asp108, and Ser112, as well as electrostatic interactions of His113 with Asp76, locks the CP-loop in a closed conformation (Fig. 3B). Upon this conformational change, Phe77 and Phe79 are placed at orientations that permit hydrophobic contacts with residues Ile55, Tyr111, Leu114, and Ala115 from the adjacent subunit, favoring the decameric assembly (Fig. 3B). Among these residues, only His113 might have its protonation state affected in the pH interval where the dimer-to-decamer transition was observed. Changes in the His113 side chain protonation probably contribute with decamer stabilization through electrostatic interactions with Asp76 at pH below 8.0.

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4A), in which the pH decrease triggers LbPrx1m decamer formation by modulating the protonation state of His$^{113}$. As the pH decreases, His$^{113}$ becomes positively charged and attracts Asp$^{76}$, located at the C$\_P$-loop. This interaction induces a conformational change in the C$\_P$-loop, locking it in a closed conformation and positioning Phe$^{77}$ and Phe$^{79}$ to create the A-type interface. As the pH increases, electrostatic interactions between His$^{113}$ and Asp$^{76}$ become weaker and LbPrx1m decamers start to dissociate into dimers. The His$^{113}$ and Asp$^{76}$ pair as well as other residues involved in the oligomerization of 2-Cys Prxs are highly conserved in Prx1 subfamily members, suggesting that the proposed padlock mechanism can be extended for most typical 2-Cys Prxs (Fig. 4B). Supporting this hypothesis, the position and orientation of Asp$^{76}$ and His$^{113}$ counterparts are fully conserved in homologous decamers, regardless the C$\_P$-redox state (Fig. 5). Moreover, the enzyme PfTrx-Px2, which crystallized as an oxidized dimer at pH 6.5 (47), has an Asn replacing the corresponding Asp$^{76}$, suggesting that lack of the Asp-His ionic pair rendered its oligomerization pH-insensitive.

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The dimer-to-decamer transition of LbPrx1m, the human Prx II (21), and the chloroplast 2-Cys Prx from Pisum sativum (7) occur in a narrow pH range between pH 7 and 8, supporting that small variations of the physiological pH can have major effects in the dimer-decamer equilibrium of typical 2-Cys Prxs in vivo. It has been reported that pH variations regulate important cellular process, such as apoptosis in mammalian cells (48) and CO$_2$ fixation in plants (23). Apoptotic stimuli induce cytosol acidification, usually of 0.4 pH units (48), whereas the dark-light transition induces a rise in the chloroplast stromal pH from 7.0 to 8.0 (23). Mitochondrial pH can vary up to 0.5 pH units (basal pH ~ 8) in mammalian cells (22). According to our results (Fig. 1) and other studies (7, 21), the dimer-decamer equilibrium of Prx1 members is responsive to such pH variations, suggesting that changes in pH homeostasis can influence the quaternary structure of cytosolic, mitochondrial, and chloroplast Prx1 members such as the human Prx II, LbPrx1m, and pea 2-Cys Prx, respectively.

The pH effect on the oligomerization of typical 2-Cys Prxs supports the hypothesis that intracellular pH affects mechanisms controlling the cellular concentration of reactive oxygen species (48). Changes in the oligomeric state of these enzymes influence both the peroxidase activity and the functional switch. The decameric form stabilizes the FF conformation

**FIGURE 3. Structural basis for the pH-induced decamerization of LbPrx1m.** Panels A and B show a structural comparison between the active sites of LbPrx1m dimer (A) and decamer (B) highlighting the C$\_P$-loop (light orange schematic) and residues (sticks) involved in the decamerization process as well as C$\_P$ (Cys$^{81}$). For clarity purposes, the C$\_P$-$\_R$ disulfide bond was omitted in both structure representations. Dashed lines represent hydrogen bonds. Labels outlined in white indicate residues from the other subunit (violet surface, panel B) that compose the A-type interface. C, SEC chromatograms of LbPrx1m at 48 $\mu$m (wt and mutants D76A (D76A) and H113A (H113A) under non-reducing (solid lines; 50 mM MMT, pH 4, 10 mM EDTA, and 100 mM NaCl) and reducing (dashed lines; 25 mM Tris-HCl, pH 7.0, 5 mM EDTA, 150 mM NaCl, and 2 mM DTT) conditions. Numbers above the graphic represent the molecular mass (kDa) of standard proteins used for column calibration. D, DLS assays of wt and the D76A mutant at 48 $\mu$m in 50 mM MMT (pH 4), 10 mM EDTA, and 150 mM NaCl. $R_m$ = hydrodynamic radius (nm), %Pd = polydispersity (%), %M = fraction of mass (%) corresponding to the peak.
required for the nucleophilic attack of the Cₚ thiolate on the peroxide substrate, rendering the sulfenylated (Cₚ-SOH) and eventually the overoxidation of Cₚ (3). Supporting this idea, 2-Cys Prx mutants unable to decamerize display lower catalytic efficiency and are less responsive to peroxide-mediated inactivation (49). Upon the disulfide bond formation between
C_{pr}-SOH and C_{pr}-SH, Prx1 subfamily decamers tend to dissociate into dimers, a process that seems to be important for the recycling step by another protein or small molecule thiol (20). For instance, recycling of rat Prx I by reduced thioredoxin is more efficient when the protein is in the dimeric state (20).

In light of these evidences and considering our findings, we suggest that pH-induced decamerrization may have a dual effect depending on the redox state of the Prx1 species. By stabilizing reduced (SH) decamers, acidification probably boosts the peroxidation step of the catalytic cycle. On the other hand, by stabilizing oxidized (SS) decamers, it might hinder the recycling step, decreasing the conversion rate of SS to SH forms. An inefficient recycling step has been associated with a lower frequency of C_{pr} peroxidation (44). The latter process has been implicated in H_{2}O_{2} signal transduction (50) and was associated with a peroxidase-to-chaperone switch (11, 12), suggesting that the pH effect on enzyme oligomerization may influence H_{2}O_{2} signaling pathways and the cell response to oxidative stress.

Despite the redox state being considered the major driving force for decamer-dimer transition, our results demonstrated that pH changes in a narrow physiological range can also be a key factor in this event. Therefore, more studies are required to shed light on how the redox state cross-talks with other oligomerization-modulating factors such as pH to regulate the structure and function of Prx1 subfamily members in vivo.

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