Kinetics of formation and reactivity of the persulfide in the one-cysteine peroxiredoxin from *Mycobacterium tuberculosis*

Received for publication, April 12, 2019, and in revised form, July 12, 2019 Published, Papers in Press, July 16, 2019, DOI 10.1074/jbc.RA119.008883

Ernesto Cuevasanta, Aníbal M. Reyes, Ari Zeida, Mauricio Mastrogiouvann, María Inés De Armas, Rafael Ratti, Beatriz Alvarez, and Madia Trujillo

From the Laboratorio de Enzimología, Instituto de Química Biólogica, Unidad de Bioquímica Analítica, Centro de Investigaciones Nucleares, Facultad de Ciencias, Departamento de Bioquímica, Facultad de Medicina, and Centro de Investigaciones Biomédicas (CEINBIO), Universidad de la República, Montevideo, Uruguay

Edited by F. Peter Guengerich

Hydrogen sulfide (H\textsubscript{2}S) participates in prokaryotic metabolism and is associated with several physiological functions in mammals. H\textsubscript{2}S reacts with oxidized thiol derivatives (i.e. disulfides and sulfenic acids) and thereby forms persulfides, which are plausible transducers of the H\textsubscript{2}S-mediated signaling effects. The one-cysteine peroxiredoxin alkyl hydroperoxide reductase E from *Mycobacterium tuberculosis* (MtAhpE–SH) reacts fast with hydroperoxides, forming a stable sulfenic acid (MtAhpE–SOH), which we chose here as a model to study the interactions between H\textsubscript{2}S and peroxiredoxins (Prx). MtAhpE–SOH reacted with H\textsubscript{2}S, forming a persulfide (MtAhpE–SSH) detectable by mass spectrometry. The rate constant for this reaction was (1.4 ± 0.2) × 10\textsuperscript{7} M\textsuperscript{-1} s\textsuperscript{-1} (pH 7.4, 25 °C), six times higher than that reported for the reaction with the main low-molecular-weight thiol in *M. tuberculosis*, mycothiol. MtAhpE was able to complete the catalytic cycle of MtAhpE and, according to kinetic considerations, it could represent an alternative substrate in *M. tuberculosis*. MtAhpE–SSH reacted 43 times faster than did MtAhpE–SH with the unspecific electrophile 4,4′-dithiopyridine, a disulfide that exhibits no preferential reactivity with peroxidatic cysteines, but MtAhpE–SSH was less reactive toward specific Prx substrates such as hydrogen peroxide and peroxynitrite. According to molecular dynamics simulations, this loss of specific reactivity could be explained by alterations in the MtAhpE active site. MtAhpE–SSH could transfer its sulfane sulfur to a low-molecular-weight thiol, a process likely facilitated by the low pKa of the leaving thiol MtAhpE–SH, highlighting the possibility that Prx participates in persulfidation. The findings of our study contribute to the understanding of persulfide formation and reactivity.

Hydrogen sulfide (H\textsubscript{2}S)\textsuperscript{5,6} has been related to the origin and evolution of life on our planet, and several organisms can produce or utilize H\textsubscript{2}S in various metabolic processes. In mammals, beyond its toxicological relevance, H\textsubscript{2}S has been associated with a variety of physiological functions, including vasodilation, neuromodulation and immunoregulation (1–3).

The pathogen *Mycobacterium tuberculosis* is the causative agent of tuberculosis disease. *M. tuberculosis* proliferates inside the phagosomes of activated macrophages, its main host cells, where it is exposed to oxidants, including hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), organic hydroperoxides (ROOH) and peroxynitrite (ONOO\textsuperscript{−}/ONOOH) (4–7). Its antioxidant defense battery includes several enzymes as well as mycothiol, the main low-molecular-weight thiol in the bacterium, with functions analogous to those of GSH. Supplementation with H\textsubscript{2}S was shown to complement the growth defects of *M. tuberculosis* strains with impaired ability to recycle mycothiol, either in cellular or animal models of disease (8). H\textsubscript{2}S produced by host cells could potentially reach the interior of *M. tuberculosis*, because it can easily cross membranes (9). Furthermore, *M. tuberculosis* produces H\textsubscript{2}S by different enzymatic mechanisms (10–13).

Among the possible reactions of H\textsubscript{2}S, those with oxidized thiol derivatives have received attention as sources of persulfides (RSSH/RSS\textsuperscript{−}).\textsuperscript{7} Indeed, hydrosulfide (HS\textsuperscript{−}), the conjugate base in equilibria with H\textsubscript{2}S, pKa\textsubscript{w} = 6.9 (14), can react with sulfenic acids (ROSO\textsubscript{2}H) and disulfides (RSSR) to produce persulfides.

---

5. The term “H\textsubscript{2}S” is used throughout the text to refer to the mixture of H\textsubscript{2}S (sulfane or hydrogen sulfide) and HS\textsuperscript{−} (sulfane or hydrogen sulfide) in rapid equilibrium at the pH of the solution, unless otherwise specified.

6. The abbreviations used are: Prx, peroxiredoxin; MtAhpE, alkyl hydroperoxide reductase E of *M. tuberculosis*; DTT, 1,4-dithiothreitol; TNB, 5-thio-2-nitrobenzoic acid; DTPpy, 4,4′-dithiopyridine; MD, molecular dynamics; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); DTPA, diethylenetriaminepentaacetic acid; PDB, Protein Data Bank.

7. In this text, “persulfide” is used for the mixture of RSSH and RSS\textsuperscript{−} in rapid equilibrium at the pH of the solution, unless otherwise specified. RSSH, usually referred to as hydrosulfide or hydrosulfite in bibliography, is named hydridodisulfide, disulfanyl or dithiohydroperoxide by IUPAC.
Formation and reactivity of persulfide in MtAhpE

Persulfides are important intermediates in sulfur metabolism in bacteria, where they are produced in enzymatic catalytic cycles (16, 17). Several enzymes, some also present in mammals, produce or transfer these functional groups; these include cystathionine γ-lyase, cystathionine β-synthase (18), mercaptopyruvate sulfurtransferase (19), sulfidequinone oxidoreductase (20, 21) and thiosulfate sulfurtransferases (22, 23). Other enzymes are able to react with persulfides; a dioxygenase can use GSH persulfide as substrate and is encoded by the ethel1 gene, which is mutated in ethylmalonic encephalopathy, a severe infantile metabolic disorder (24). Recently proposed as intermediates in the transduction of the signaling effects observed after the administration of H2S (25, 26), persulfides have been generating increased interest. According to the hypothesis of persulfide-mediated signaling, the formation of a persulfide in certain cysteines could unleash changes in the activity of effector proteins, like the inhibition of papain, a cysteine-dependent protease (27), PTEN, a lipid phosphatase (28), and aquaporin-8, a membrane channel (29), among others. Possible roles in regulation and catalysis are still being explored, and the reactivity and physicochemical features of these species are poorly understood. Lately, some molecular models have been proposed and analytical methods have been developed to study persulfides both in vivo and in vitro (15, 30–38). When thiols are modified to persulfides, nucleophilicity is maintained and probably increased due to two factors: (a) increased acidity with respect to thiols (39), which results in increased availability of the deprotonated, more nucleophilic form at neutral pH; and (b) the α effect, i.e. the enhanced reactivity of a nucleophilic atom when it is adjacent to an atom containing one or more unshared pairs of electrons (40). In addition, a new property is acquired: electrophilicity. The reduction and the oxidation of persulfides are also possible; either H2S and thiols or perthiosulfenic acids (RSSOH) and polysulfides are produced, respectively. The high reactivity of persulfides determines the instability of these compounds in aqueous solutions (35), limiting their study and highlighting the importance of developing suitable models.

Peroxiredoxins (Prxs) are a family of antioxidant enzymes that play crucial roles in redox signaling (41–43). These enzymes are thiol-dependent peroxidases with ping-pong kinetic mechanisms. The oxidizing substrate (H2O2, organic hydroperoxide or peroxyxynitrite) reacts with the thiolate at the peroxidatic cysteine in the reduced enzyme to form a sulfenic acid (44). The reactivities of the thiolates in peroxidatic cysteines of Prx with hydroperoxides are several orders of magnitude faster than those of typical low- or high-molecular-weight thiols. This can be explained by the decrease in the energy of activation of the reaction by an exquisite network of electrostatic and hydrogen-bonding interactions involving the functional groups of an arginine and a threonine among others (45–47). Besides, the environment of the peroxidatic cysteine lowers the pKa of the thiol by several units relative to free cysteine (48). Once oxidized, the sulfenic acid is then reduced back to thiol by the reducing substrate(s), either directly or after a resolution step that involves the formation of a disulfide bond with a second cysteine residue (resolving cysteine), depending on the Prx subfamily (44, 49). Often, a thioredoxin/thioredoxin reductase system reduces the disulfide bond to complete the catalytic cycle (44). The direct reduction of the sulfenic acid occurs in the so-called one-cysteine Prxs, such as alkyl hydroperoxide reductase E of M. tuberculosis (MtAhpE). This Prx catalyzes the reduction of several hydroperoxides, being most active with peroxyxynitrite and fatty acid hydroperoxides (50, 51). The sulfenic acid of MtAhpE (MtAhpE–SOH) is reduced by the glutaredoxin-like protein mycoredoxin-1, either directly or after formation of a mixed disulfide with mycothiol (52–54). H2S is another possible reducing substrate for MtAhpE–SOH; however, it is not clear how effective its contribution could be. Moreover, both the ability of the resulting persulfide (MtAhpE–SSH) to react with typical Prx substrates or, alternatively, the capacity to be transferred to acceptor thiols remain unexplored.

In this work, MtAhpE was chosen as a model for persulfidation studies because this one-cysteine Prx presents the advantage that its sulfenic acid is relatively stable (52). We focused on the kinetic characterization of the reaction between MtAhpE–SOH and H2S to form a persulfide. Kinetic methods were employed to assess the possibility that H2S could act as a reducing substrate of the sulfenic acid and the relative contribution with respect to the better characterized mycobacterial reducing systems (mycothiol and mycoredoxin-1) is discussed. To compare the reactivity of the persulfide in the peroxidatic cysteine to that of the thiol, we evaluated the kinetics with specific substrates and unspecific reactants of Prxs. Additionally, we performed computational simulations to analyze the structural basis of the effects observed. Furthermore, the possibility of Prx assistance in persulfidation reactions (transpersulfidation) was explored.

Results

Detection of the persulfide in MtAhpE

The formation of the persulfide from the reaction of H2S with MtAhpE–SOH was revealed by the detection of its alkylation product after treatment with iodoacetamide and by the reduction of this product to MtAhpE–SH with DTT (Fig. 1A). Cysteine modifications to MtAhpE–SSH and sulfenic acid (MtAhpE–SO2H) involve similar mass shifts. The use of an alkyllating agent allows us to distinguish unequivocally the nature of the product, because only persulfides will be alkylated due to their high nucleophilicity in opposition to the poor one of sulfenic acids. Furthermore, alkylated persulfides are characterizedly reduced by thiol-containing compounds like DTT (55). A species with a molecular mass of 19,408 Da, consistent with the S-carbamidomethyl derivative of the peroxidatic cysteine persulfide (MtAhpE–SS–CAM), was detected in equimolar mixtures of MtAhpE–SH (19,319 Da), H2S and H2O2 at different incubation times. The species was already present when iodoacetamide was added 30 s after mixing, and maxi-
mum yields were obtained after 2–5 min (Fig. 1B). MtAhpE–SSH was relatively stable, because it could still be detected after incubation times before alkylation of 15 and 30 min (Fig. S1), although the signal of the MtAhpE–SS–CAM derivative decreased while that corresponding to reduced MtAhpE (MtAhpE–S–CAM, 19,376 Da) increased. No alkylated MtAhpE polysulfide derivatives (i.e. MtAhpE–SSnS–CAM, with n ≥1) were detected under these experimental conditions. MtAhpE–SS–CAM, an unsymmetrical disulfide, was reduced by treatment with DTT to form the original thiol (19,319 Da) (Fig. 1C). Additionally, a peak corresponding to 19,352 Da was detected in all samples and was particularly evident in those where MtAhpE–SH was incubated with H2O2 and H2S. Because this species remained after DTT addition, it most probably reflects the presence of protein over-oxidized to MtAhpE–SO2H.

Kinetics of the reaction of H2S with MtAhpE–SOH

As shown above, H2S is able to react with MtAhpE–SOH forming a persulfide. With the aim of evaluating the viability of this reaction among alternative reducing systems, the kinetics of the reaction was studied. Because direct measurements of concentration changes are not straightforward in this time scale, determinations were performed by two competition assays. The first one was a competition between H2S and 5-thio-2-nitrobenzoic acid (TNB) for MtAhpE–SOH (Fig. 2A). The incubation of MtAhpE–SOH with the colored thiol TNB under pseudo-first-order conditions in the absence of H2S (Fig. 2B, blue trace) reproduced previous observations and confirmed the second-order rate constant of the direct reaction between MtAhpE–SOH and TNB to be $(2.2 \pm 0.1) \times 10^3 \text{M}^{-1} \text{s}^{-1}$ (50). The reaction yielded a mixed disulfide (MtAhpE–S–TNB) that slowly reacted with a second molecule of TNB regenerating the reduced enzyme, as reported previously (50). When MtAhpE–SOH was mixed with TNB in the presence of H2S, the observed exponential rate constants of TNB decay ($k_{obs}$) increased linearly with the concentration of H2S while the amplitudes decreased (Fig. 2B and C), as expected for competition kinetics (56). A second-order rate constant of $(1.4 \pm 0.2) \times 10^3 \text{M}^{-1} \text{s}^{-1}$ for the reaction of H2S with MtAhpE–SOH (pH 7.4, 25 °C) was obtained from this competition assay. The MtAhpE–S–TNB product reacted with excess H2S and produced TNB, explaining the linear increases in absorbance after the exponential decays of TNB in Fig. 2B.

A second approach for determining the kinetics of the reaction of H2S with MtAhpE–SOH consisted of a competition assay with the over-oxidation reaction of MtAhpE–SOH to MtAhpE–SO2H following the changes in the protein intrinsic fluorescence emission that occur when the enzyme is exposed to excess H2O2 (50–52). The fast oxidation of MtAhpE–SH produced a rapid decrease in its fluorescence emission due to the formation of the MtAhpE–SOH during the first s, which is consistent with the reported rate constant of $8.2 \times 10^4 \text{M}^{-1} \text{s}^{-1}$ (50). A second phase of the reaction showed a slow recovery of the emission due to enzyme over-oxidation to MtAhpE–SO2H, as shown previously (50–52). The presence of H2S produced changes in the amplitude and in the observed rate constants of this second phase (Fig. 3). The global fitting of the fluorescence changes according to a simple competition model...
Formation and reactivity of persulfide in MtAhpE

Figure 2. Competition assay of H2S and TNB toward MtAhpE–SOH. A, reactions of the competition assay with TNB. The species are represented in their protonated state for simplicity. B, time courses for the absorbance of TNB (30 μM) when mixed with MtAhpE–SOH (1.4 μM) and different concentrations of Na2S in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C). The gray trace represents the best fit to an exponential plus straight-line function \(\text{Abs}_{412\text{ nm}} = A \cdot \exp(-k_{\text{obs}} \cdot t) + b \cdot t + c\), where \(A\) is the amplitude of the exponential contribution; \(k_{\text{obs}}\) is the observed exponential rate constant; \(b\) is the slope of the linear contribution; and \(c\) is the offset. The data used for the fits corresponded to 10 half-lives minus the first second. C, exponential rate constants obtained from the exponential phase of absorbance decay versus Na2S concentration. Representative results of an experiment performed three independent times are shown.

The study of the reactivity of both thiol and persulfide and is not expected to establish specific interactions with the active site. A mixture of MtAhpE–SOH and H2S was aged for increasing time periods and mixed with DTDPy under pseudo-first-order conditions (Fig. 4). The 4-thiopyridone released by reaction of DTDPy with H2S and MtAhpE–SSH was followed at 324 nm, and the kinetics showed a biphasic profile. The fast phase, attributable to the reaction of MtAhpE–SSH with DTDPy, increased its amplitude during the first 5 min while MtAhpE–SSH was being slowly produced, as expected from the concentrations of MtAhpE–SOH and H2S, and the rate constant of \((1.4 \pm 0.2) \times 10^3 \text{ M}^{-1} \text{s}^{-1}\) reported in the previous section. After \(~400\) s, the amplitude of the fast phase became constant, showing that all persulfide had been formed and remained stable during at least 30 min under these experimental conditions (Fig. 4B). From the amplitude of the fast phase, the amount of MtAhpE–SSH formed was calculated as 1.1 μM, representing 81% of total MtAhpE. The slow phase that decreased in amplitude with time was attributed to the reaction of H2S with DTDPy and had a rate constant of \(545 \pm 42 \text{ M}^{-1} \text{s}^{-1}\) at pH 7.4 and 25 °C. Given that at \(~900\) s the formation of MtAhpE–S–SH had reached a plateau while H2S had been depleted, experiments with varying concentrations of DTDPy were performed after this aging period. The observed rate constants for the fast phase increased linearly with DTDPy concentration (Fig. 4C). From the slope, the second-order rate constant for the reaction of MtAhpE–SSH with DTDPy was calculated to be \((1.8 \pm 0.1) \times 10^3 \text{ M}^{-1} \text{s}^{-1}\) at pH 7.4 and 25 °C. In comparison, the rate constant for the reaction of the thiol MtAhpE–SH with DTDPy was \(42 \pm 8 \text{ M}^{-1} \text{s}^{-1}\) under the same conditions (Fig. 4C).

Reactivity of MtAhpE–SSH with an unspecific target, the disulfide DTDPy

The relative stability of the persulfide derivative of this Prx facilitated the study of its reactivity. The kinetics of the reaction of the persulfide toward the electrophile DTDPy was evaluated. DTDPy is a synthetic disulfide used as a reporter, which allows the study of the reactivity of both thiol and persulfide and is not expected to establish specific interactions with the active site. A mixture of MtAhpE–SOH and H2S was aged for increasing time periods and mixed with DTDPy under pseudo-first-order conditions (Fig. 4). The 4-thiopyridone released by reaction of DTDPy with H2S and MtAhpE–SSH was followed at 324 nm, and the kinetics showed a biphasic profile. The fast phase, attributable to the reaction of MtAhpE–SSH with DTDPy, increased its amplitude during the first 5 min while MtAhpE–SSH was being slowly produced, as expected from the concentrations of MtAhpE–SOH and H2S, and the rate constant of \((1.4 \pm 0.2) \times 10^3 \text{ M}^{-1} \text{s}^{-1}\) reported in the previous section. After \(~400\) s, the amplitude of the fast phase became constant, showing that all persulfide had been formed and remained stable during at least 30 min under these experimental conditions (Fig. 4B). From the amplitude of the fast phase, the amount of MtAhpE–SSH formed was calculated as 1.1 μM, representing 81% of total MtAhpE. The slow phase that decreased in amplitude with time was attributed to the reaction of H2S with DTDPy and had a rate constant of \(545 \pm 42 \text{ M}^{-1} \text{s}^{-1}\) at pH 7.4 and 25 °C. Given that at \(~900\) s the formation of MtAhpE–S–SH had reached a plateau while H2S had been depleted, experiments with varying concentrations of DTDPy were performed after this aging period. The observed rate constants for the fast phase increased linearly with DTDPy concentration (Fig. 4C). From the slope, the second-order rate constant for the reaction of MtAhpE–SSH with DTDPy was calculated to be \((1.8 \pm 0.1) \times 10^3 \text{ M}^{-1} \text{s}^{-1}\) at pH 7.4 and 25 °C. In comparison, the rate constant for the reaction of the thiol MtAhpE–SH with DTDPy was \(42 \pm 8 \text{ M}^{-1} \text{s}^{-1}\) under the same conditions (Fig. 4C).
 Formation and reactivity of persulfide in MtAhpE

Figure 4. Reactivity of MtAhpE–SSH toward the electrophile DTPy. A, persulfide was prepared by incubation of MtAhpE–SSH (1.30 μM) with H2O2 (1.29 μM) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C) during 3 min. Then, Na2S (1.29 μM) was added and mixed with DTPy (123 μM) every 1.5 min in a stopped-flow instrument. The gray trace represents the best fit to an exponential plus straight-line function (Abs324 nm = Aexp(−kexp, t) + bτ + c, where A is the amplitude of the exponential contribution; kexp is the observed rate constant; b is the slope of the linear contribution; and c is the offset). B, amplitude of experiments performed as in A but with increasing concentrations of DTPy. The variations in the amplitude of the fast phase reveal the timing for the formation of MtAhpE–SSH. C, after aging mixtures of MtAhpE–SSH, H2O2, and Na2S for ~900 s, samples were mixed with varying concentrations of DTPy, and the observed rate constants of the fast phase were determined. From the linear correlation with the concentration of DTPy, a second-order rate constant of (1.8 ± 0.1) × 104 M−1 s−1 was obtained for the persulfide (red circles). The observed rate constants for the reaction of MtAhpE–SSH with DTPy are shown in black circles (42 ± 8 M−1 s−1). The rate constant values are the mean and standard deviation of three independent experiments.

Reactivity of MtAhpE–SSH toward the specific Ppx substrates, peroxynitrite and H2O2

Despite the increased intrinsic reactivity of the persulfide with respect to the thiol, the ability of MtAhpE–SSH to consume specific substrates of the enzyme, which implies additional factors, remained to be evaluated. The decay of peroxynitrite (12 μM) was highly accelerated in the presence of 15 μM reduced MtAhpE–SSH (Fig. 5A, black trace) and occurred mostly in the mixing time of the apparatus (1.1 ms), in agreement with the fast reaction previously reported (1.7 × 107 M−1 s−1 at pH 7.4 and 25 °C (50)). On the contrary, both MtAhpE–SOH and MtAhpE–SSH caused only modest increases in peroxynitrite decay rate (Fig. 5A, blue and red traces, respectively). Considering ~80% MtAhpE–SSH formation yield, as determined above (Fig. 4A), the rate constant of the reaction with peroxynitrite was estimated as ~104 M−1 s−1 at pH 7.4 and 25 °C.

In the case of H2O2, its consumption by MtAhpE–SSH (45 μM) was almost 1:1 during the first 30 s, as already described (51), and in agreement with the reported rate constant of 8.2 × 104 M−1 s−1 at pH 7.4 (Fig. 5B, black squares) (50). In the presence of MtAhpE–SOH (Fig. 5B, blue circles), the decay of H2O2 was slower, and from the initial rate of H2O2 decay a rate constant of 78 M−1 s−1 was estimated, which agrees with the low rate constant of the reaction between MtAhpE–SOH and H2O2 previously determined (40 M−1 s−1) (50). The rate of H2O2 consumption by MtAhpE–SSH (Fig. 5B, red triangles) was similar to that of MtAhpE–SOH. From the initial rate of H2O2 decay and considering persulfide formation yields of ~80%, the rate constant of MtAhpE–SSH oxidation by H2O2 was estimated to be 109 M−1 s−1 at pH 7.4 and 25 °C.

Catalytic consumption of H2S and H2O2 by MtAhpE

To study whether H2S is able to complete the catalytic cycle of the enzyme, MtAhpE–SSH was added to a solution containing H2S and H2O2, and aliquots were removed to determine H2S as well as H2O2 consumption during the incubation. Although H2O2 is able to consume H2S in the absence of catalysts, in agreement with the slow uncatalyzed reaction already reported (0.35 M−1 s−1 at pH 7.4 and 25 °C (57)), increased rates of H2S and H2O2 consumption were observed when substoichiometric amounts of the enzyme were included (Fig. 6, A and B). The initial rates of H2S decay showed a linear dependence on the concentration of MtAhpE–SSH with a slope 2.2 × 10−2 s−1 at
were done in 0.05 M phosphate buffer (pH 7.4, 25 °C), and the H2O2 consuming activity in the presence of H2O2 (Fig. 6).

The concentration of H2S was determined using the ferrous oxidation–xylenol orange method. The initial rates of H2S consumption obtained at different concentrations of the assayed substrate confirm the H2S-consumption activity in the presence of H2O2 (Fig. 6).

The transfer of the sulfane sulfur of MtAhpE–SSH to a thiol probe with cysteine sulfenic acids (15, 36). The peroxidatic cysteines in Prxs constitute preferential targets for hydroperoxides due to the high reactivity and cellular abundance (63, 64). Their reaction constitutes a source of sulfenic acids, which are then reduced by several pathways. The feasibility of the reaction of H2S with a sulfenic acid is determined by kinetic aspects and is favored when the latter is long-lived. We particularly focused on MtAhpE–SH, which produces a relatively stable sulfenic acid due to the absence of thiols in the vicinity of the active site (52).

The values obtained for MtAhpE–SSH can be compared with those obtained for the reaction of the sulfenic acid in human serum albumin (HSA–SOH) with H2S (15). The rate constant for HSA–SOH was calculated to be 1.4 × 10^3 M^-1 s^-1 at pH 7.4 and 25 °C. Considering that the reactive species are protonated MtAhpE–SOH and ionized HS^- (65) and because the reported pK_a values are 6.6 for MtAhpE–SOH (50) and 6.9 for H2S (14), the pH-independent rate constant can be calculated to be 1.4 × 10^3 M^-1 s^-1.

The values obtained for MtAhpE–SSH were determined to be (1.4 ± 0.2) × 10^3 M^-1 s^-1 at pH 7.4 and 25 °C. Considering that the reactive species are protonated MtAhpE–SOH and ionized HS^- and because the reported pK_a values are 6.6 for MtAhpE–SOH (50) and 6.9 for H2S (14), the pH-independent rate constant can be calculated to be 1.4 × 10^3 M^-1 s^-1.

The values obtained for MtAhpE–SSH can be compared with those obtained for the reaction of the sulfenic acid in human serum albumin (HSA–SOH) with H2S (15). The rate constant for HSA–SOH was calculated to be 1.4 × 10^3 M^-1 s^-1 at pH 7.4

8 k_pH = k_pH-incl × ([K_a_H2S]/[K_a_H2S + [H^+]]) × ([H^+]/[K_a_MtAhpE_SOH + [H^+]]).

In summary, MD simulations suggest that persulfidation of MtAhpE leads to a significant disruption in the topology of the active site that alters key interactions involved in catalysis.

**Transferring the sulfane sulfur of MtAhpE–SSH to a thiol probe**

With the aim of determining whether MtAhpE–SSH is able to transfer its sulfane sulfur to other thiols, MtAhpE–SSH was incubated with sulfane sulfur probe 4 (SSP4), a nonfluorescent thiol-containing compound used as a probe for sulfane sulfurs. The transfer of sulfane sulfur to a thiol of the probe leads to the formation of a persulfide, which undergoes spontaneous cyclization to release a fluorophore (61, 62). Incubation of SSP4 with MtAhpE–SSH led to an increase in the fluorescence emission (Fig. 8). This increase occurred to a much larger extent than in controls with MtAhpE–SH or MtAhpE–SOH. According to calibration curves using Na2S2, the yield of the transsulfuration process was 95 ± 5%.

**Discussion**

A plausible fate of H2S in cells is represented by its reactions with cysteine sulfenic acids (15, 36). The peroxidatic cysteines in Prxs constitute preferential targets for hydroperoxides due to the high reactivity and cellular abundance (63, 64). Their reaction constitutes a source of sulfenic acids, which are then reduced by several pathways. The feasibility of the reaction of H2S with a sulfenic acid is determined by kinetic aspects and is favored when the latter is long-lived. We particularly focused on MtAhpE–SH, which produces a relatively stable sulfenic acid due to the absence of thiols in the vicinity of the active site (52).

The values obtained for MtAhpE–SSH were determined to be (1.4 ± 0.2) × 10^3 M^-1 s^-1 at pH 7.4 and 25 °C. Considering that the reactive species are protonated MtAhpE–SOH and ionized HS^- (65) and because the reported pK_a values are 6.6 for MtAhpE–SOH (50) and 6.9 for H2S (14), the pH-independent rate constant can be calculated to be 1.4 × 10^3 M^-1 s^-1.

The values obtained for MtAhpE–SSH can be compared with those obtained for the reaction of the sulfenic acid in human serum albumin (HSA–SOH) with H2S (15). The rate constant for HSA–SOH was calculated to be 1.4 × 10^3 M^-1 s^-1 at pH 7.4

8 k_pH = k_pH-incl × ([K_a_H2S]/[K_a_H2S + [H^+]]) × ([H^+]/[K_a_MtAhpE_SOH + [H^+]]).
and 25 °C, whereas the pH-independent rate constant was 4.10^1 M/s (assuming that most HSA–SOH was protonated at pH 7.4). Thus, it can be concluded that MtAhpE–SOH is 30 times more reactive than HSA–SOH with HS^−.

Substoichiometric concentrations of MtAhpE were able to consume H_2S and H_2O_2 catalytically, suggesting that MtAhpE–SH can be regenerated and that the enzyme can initiate a new catalytic cycle. As precedent, it was proposed that bovine Prx6, another one-cysteine Prx from a different subfamily, is able to consume H_2O_2 using H_2S as a reducing substrate via the formation of a persulfide in its peroxidatic cysteine (66). In contrast, in Prx6 from Arenicola marina, no evidence could be obtained for H_2S participation in the catalytic cycle (67). In the case of MtAhpE, the catalysis in the presence of excess H_2S and H_2O_2 could proceed by a variety of pathways. It surely starts with the fast oxidation of MtAhpE–SH by H_2O_2 (8.2 ± 10^4 M/s, pH 7.4 (50), Equation 3) to produce MtAhpE–SOH, followed by the reaction with H_2S to form MtAhpE–SSH ((1.4 ± 0.2) ± 10^3 M/s, pH 7.4, Equation 4). Then, MtAhpE–SSH can react with H_2S to recover the original thiol and produce HSSH and other polysulfide by-products (Equation 5). Alternatively, MtAhpE–SSH can react with H_2O_2 to form a perthiosulfenic acid (RSSOH) and other higher oxidation states (RSSO_2H and RSSO_3H), which can then be reduced to thiol or persulfide by H_2S. The favored pathway is determined by kinetic aspects that remain to be elucidated. Nevertheless, the slope of the plot of rate versus enzyme concentration indicated a turnover of 2.2 ± 10^−2 s^−1 at the used H_2S and

Figure 7. Structural changes upon persulfidation in MtAhpE’s active site. A, structural alignment of representative snapshots of the MtAhpE dimer obtained from MD simulations with Cys45 as thiolate (black) or as persulfide (red). B, comparison of thiolate and persulfide in Cys45 depicting residues Thr42, Cys45 and Arg116. C, distribution of α-helix and β-sheet content (%) for both MD simulations. D, comparison of root mean square fluctuations (Å) in a per-residue basis calculated from thiolate in Cys45 (black) and persulfide in Cys45 (red) MD simulations. E, distribution of relevant active-site interactions. Selected distances are the same as highlighted in B.

Figure 8. Transfer of the sulfane sulfur of MtAhpE–SSH to the thiol in the SSP4 probe. MtAhpE–SOH was prepared by incubation of MtAhpE–SH (4 μM) with H_2O_2 (4 μM) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C) during 2 min. The persulfide was formed by adding Na_2S (4 μM) to sulfenic acid preparations and aging during 15 min, time in which the reaction is expected to be completed. Then, samples were diluted 2-fold; SSP4 (20 μM) was added, and fluorescence emission was followed for MtAhpE–SH (black), MtAhpE–SOH (blue), MtAhpE–SSH (red) and buffer (green). Traces are the average of four runs from two independent experiments performed in duplicates.

Formation and reactivity of persulfide in MtAhpE
Formation and reactivity of persulfide in MtAhpE

H$_2$O$_2$ concentrations (Fig. 6C). Assuming that the rate-limiting step is a second-order reaction, either the reaction of MtAhpE–SSH with H$_2$S or with H$_2$O$_2$, and considering that the concentrations of H$_2$S and H$_2$O$_2$ were 125 and 127 $\mu$M, respectively, it can be calculated that the rate constant for the rate-limiting second-order reaction is $\sim$170 m$^{-1}$ s$^{-1}$. Clearly, the reaction of MtAhpE–SOH with H$_2$S, which has an 8-fold higher rate constant ((1.4 $\pm$ 0.2) $\times$ 10$^3$ m$^{-1}$ s$^{-1}$, pH 7.4), is not rate-limiting in the catalytic process. Besides, the rate of the reaction of MtAhpE–SSH with H$_2$O$_2$ ($\sim$109 m$^{-1}$ s$^{-1}$) is below the expected rate-limiting step, leading to the reaction in Equation 5 as the most likely to participate in the catalytic cycling.

\[
\text{RSH} + \text{H}_2\text{O}_2 \rightarrow \text{RSOH} + \text{H}_2\text{O} \quad \text{(Eq. 3)}
\]

\[
\text{RSOH} + \text{H}_2\text{S} \rightarrow \text{RSSH} + \text{H}_2\text{O} \quad \text{(Eq. 4)}
\]

\[
\text{RSSH} + \text{H}_2\text{S} \rightarrow \text{RSH} + \text{HSSH} \quad \text{(Eq. 5)}
\]

In cellular contexts, the reaction of H$_2$S with sulfenic acids could be of relevance in one-cysteine Prxs, where the resolving cysteine is absent and the sulfenic acid could be long-lived. It could also be relevant in eukaryotic two-cysteine Prx, particularly in those cases where the reaction of the resolving cysteine with the sulfenic acid to form a disulfide is relatively slow so that the sulfenic acid would have a significant half-life, and that is the case of eukaryotic typical two-cysteine Prxs in opposition to bacterial counterparts (68).

The second-order rate constant of the reaction of MtAhpE–SOH with H$_2$S ((1.4 $\pm$ 0.2) $\times$ 10$^3$ m$^{-1}$ s$^{-1}$) is six times higher than that reported for the reaction of MtAhpE–SOH with mycothiol and is similar to that reported for mycoredoxin-1 (237 and 1.6 $\times$ 10$^2$ m$^{-1}$ s$^{-1}$, respectively (52)), which are the endogenous substrates in _M. tuberculosis_ known up to date. The main fate of MtAhpE–SOH in cells is dictated not only by kinetic constants but also by the concentration of the targets. Although reports on the steady-state concentrations of mycoredoxin-1 in _M. tuberculosis_ are still lacking, levels of both mycothiol (1–8 mM (69)) and H$_2$S (~370 mM (69, 70)) have been estimated. Thus, H$_2$S could represent an effective substrate in _M. tuberculosis_, an alternative to the mycothiol and mycoredoxin-1. The mechanisms of regulation of mycothiol and H$_2$S synthesis in the bacterium are only starting to be unraveled (71, 72); therefore, further work is required to establish their relative contribution for MtAhpE reduction during different metabolic conditions. Furthermore, the roles of H$_2$S and mycothiol/mycoredoxin-1 as electron donors for AhpE and AhpE-like proteins expressed in other Actinomycetes (cbs.wfu.edu/prx/test/prxInfo.php?subfamily=6) (94), which differ in mycothiol content (70) and can be exposed, depending on their habitat, to high H$_2$S concentrations, deserve further investigation. Indeed, H$_2$S supplementation was shown to complement the growth defect of bacterial strains with decreased ability to regenerate the reduced form of mycothiol (8). In addition, it was shown that Rv2238c, the gene encoding MtAhpE, is transcriptionally up-regulated in a cellular model of intraocular tuberculosis (73). Our study gives insights into possible mechanisms of cross-talk between the pathogen and its host at a junction between H$_2$S signaling and the antioxidant defense systems.

### Table 1

| Substrate | $K$ (m$^{-1}$ s$^{-1}$) |
|-----------|-----------------------|
| DTPPy     | ONOOH     |
| MtAhpE–SH | 42 $^{a,b}$ | 1.9 $\times$ 10$^{-4}$ $^{(50)}$ | 8.2 $\times$ 10$^{-4}$ $^{(50)}$ |
| MtAhpE–SSH| 1.8 $\times$ 10$^{-4}$ $^{(50)}$ | $\ldots$ $^{a}$ | $\ldots$ $^{a}$ |
| HSA–SSH   | 7.6 $\times$ 10$^{-4}$ $^{(15)}$ | 270 $\times$ 10$^{-4}$ $^{(15)}$ | 2.1 $\times$ 10$^{-4}$ $^{(74)}$ |
| HSA–SH    | 1.7 $\times$ 10$^{-4}$ $^{(15)}$ | 1.2 $\times$ 10$^{-4}$ $^{(15)}$ | ND $^{a}$ |

* $^{a}$ Data were determined at 25 °C and pH 7.4.
* $^{b}$ Data were determined in this work.
* $^{c}$ Data were determined at 20 °C and pH 7.4.
* $^{d}$ Data were determined at 37 °C and pH 7.4.
* ND means not determined.

The reactivity of MtAhpE–SSH toward an unspecific electrophilic target was probed using DTPPy. This synthetic disulfide was chosen because it has high intrinsic reactivity, because the reaction can be followed through the absorbance of 4-thiopyridone, because it can be used in pseudo-first–order excess so that the concentration of persulfide does not need to be exactly known, and because it has been used with HSA before (15). In addition, it constitutes an unspecific “substrate” for MtAhpE, which would allow us to interrogate thiol and persulfide reactivity in the absence of specificity aspects. Apparent second–order rate constants of (1.8 $\pm$ 0.1) $\times$ 10$^3$ m$^{-1}$ s$^{-1}$ and 42 $\pm$ 8 m$^{-1}$ s$^{-1}$ were obtained at pH 7.4 for MtAhpE–SSH and for MtAhpE–SH, respectively. The value obtained for the persulfide was 43 times higher than that for the thiol. Considering that the reactive species are ionized, because the $K_p$ of MtAhpE–SH is 5.2 ($^{50}$), and the $K_p$ of MtAhpE–SSH is also likely to be much lower than 7.4 (39), the values obtained at pH 7.4 are likely to reflect pH-independent values. Thus, the increased reactivity of MtAhpE–SSH with respect to MtAhpE–SH cannot be ascribed to changes in availability of the ionized species. Rather, they can be ascribed to an increase in intrinsic reactivity. A previous publication (15) reported rate constants for the reaction of DTPPy with the persulfide (HSA–SSH) and the thiol (HSA–SH) in human serum albumin as (1.7 $\pm$ 0.1) $\times$ 10$^4$ m$^{-1}$ s$^{-1}$ and (7.6 $\pm$ 0.4) $\times$ 10$^2$ m$^{-1}$ s$^{-1}$, respectively, at pH 7.4 and 25 °C (Table 1). These values translate into pH-independent rate constants of $\sim$2 $\times$ 10$^{8}$ m$^{-1}$ s$^{-1}$ for HSA–SSH and 7 $\times$ 10$^8$ m$^{-1}$ s$^{-1}$ for HSA–SH, which has a $K_p$ of 8.1 (74). Thus, the formation of a persulfide produced a 20-fold increase in the reactivity at pH 7.4 and just a 3-fold increase in pH-independent rate constants. The increase in intrinsic reactivity with DTPPy of the persulfide relative to the thiolate can be due to the $\alpha$-effect, to changes in solvation, to alterations in weak interactions in the environment of the cysteine or to combinations of these effects.

Remarkably, the reactivity of MtAhpE–SH toward DTPPy at pH 7.4 was 1 order of magnitude lower than that of HSA–SH. In contrast, the reactivity toward hydroperoxides, the specific substrates of Prxs, is several orders of magnitude higher for MtAhpE–SH than for HSA–SH (Table 1). This is another example of the low reactivity of Prxs toward nonspecific compounds and contributes to the concept that there is no such thing as a general reactive cysteine (46, 75). The peroxidatic cysteine microenvironment in Prxs specifically accelerates the reaction with their hydroperoxide substrates.

In contrast to the increased reactivity toward the synthetic disulfide DTPPy of MtAhpE–SSH versus MtAhpE–SH, the
reactivity of MtAhpE–SSH toward H₂O₂ and peroxynitrite was several orders of magnitude lower than that of MtAhpE–SH. The mild reactivity of MtAhpE–SSH with these specific Prx substrates appears to be an effect of geometrical distortion of the catalytic site, which seems to fit the requirements for the correct interaction of hydroperoxides with the peroxodicysteine in the thiolate but not the persulfide state, through hydrogen bonds with Arg116 and Thr42 during both the formation of the substrate complex and the transition state (45). In addition to changing the reactivity of this site due to shifts in distances, the formation of a persulfide could also change the value of the pKₐ. Although the acidity appears to be lower in low-molecular-weight persulfides with respect to their analogous thiols, it is not easy to predict the persulfide pKₐ in the case of a Prx because of the special environment of the active site. Furthermore, persulfides are expected to improve the reactivity as soft bases (15), which make them more likely to react with sulfides than with hard peroxides.

Once formed in a Prx, what fate could the persulfide have? Although further reaction with H₂S or H₂O₂ can occur in vitro, it is likely that reactions with thiols predominate, considering the high cellular concentrations of low- and high-molecular-weight thiols. In fact, proteins of the thioredoxin and glutaredoxin families have been shown to react with persulfides (36, 37). The possibility of direct attack of a protein thiolate in the outer sulfur of a Prx persulfide would be promoted by the relatively high acidity of the leaving group thiol. The result would be the formation of a persulfide in the attacking protein. This would constitute a mechanism for persulfidation that could contribute to the relatively high levels of persulfidation that have been detected (36–38). As proof of concept, MtAhpE–SSH was able to transfer the persulfide to a low-molecular-weight thiol in high yield (Fig. 8). Thus, the reaction of H₂S with Prx sulfenic acids shown in this study opens up the possibility of Prx participation in the persulfidation of proteins.

Experimental procedures

Chemicals

Sodium sulfide (Na₂S·9H₂O) was obtained from either J. T. Baker or Carlo Erba. H₂O₂ was obtained from Mallinkrodt Chemicals. 5,5’-Dithiobis-(2-nitrobenzoic acid) (DTNB), 1,4-dithiothreitol (DTT), iodoacetamide and diethylenetriamine-pentaacetic acid (DTPA) were purchased from Sigma. 4,4’-Di-thiodipyridine (DTDPy) was purchased from Acros Organics. SSP4 and Na₂S₂ were obtained from Dojindo Molecular Technologies.

Preparation of reagents

H₂S solutions were prepared by dissolving Na₂S·9H₂O in extensively degassed distilled water plus 0.1 mM DTPA in sealed vials and used immediately. H₂O₂ was prepared by dilution of stock solutions in ultrapure water and quantified by measuring absorbance at 240 nm (ε₂₄₀ = 39.4 M⁻¹ cm⁻¹) (76). Peroxynitrite was synthesized from H₂O₂ and nitrous acid as described previously and treated with granular manganese dioxide to eliminate residual H₂O₂ (77). Nitrite contamination was typically <30% of peroxynitrite concentration. Peroxynitrite concentrations were determined spectrophotometrically at 302 nm (1705 M⁻¹ cm⁻¹) (78). Solutions of Na₂S₂ were prepared in ultrapure water immediately before use. Solutions of 5-thio-2-nitrobenzoic acid (TNB) were prepared as described previously (79). DTDPy stock solutions were prepared in 95% ethanol. SSP4 was diluted in DMSO.

Protein expression and purification

MtAhpE (encoded by the gene Rv2238c, https://mycobrowser.epfl.ch) (95) was expressed in Escherichia coli BL21(DE3) (expression vector pDEST17) as a recombinant His-tagged protein and purified as described previously (80). The concentration of the protein subunits of this homodimeric enzyme was determined spectrophotometrically at 280 nm, ε₊₁₀₀ = 3,950 M⁻¹ cm⁻¹ calculated according to protein sequence using the ProtParam tool in ExPASy, http://web.expasy.org/protparam (81).

Protein thiol reduction and quantitation

MtAhpE was reduced immediately before use by incubation with 2 mM DTT for 30 min at 4 °C. Excess reductant was removed by gel filtration using a HiTrap desalting column (Amersham Biosciences) and UV detection at 280 nm. Protein thiol content was measured by reaction with either DTNB (ε₁₁₀ = 14,150 M⁻¹ cm⁻¹) (82) or DTDPy (ε₁₂₀ = 21,400 M⁻¹ cm⁻¹) (83). As expected, the reduced enzyme contained one thiol per protein subunit.

Preparation of MtAhpE derivatives

MtAhpE–SOH was obtained by treatment of the reduced enzyme with an equivalent amount of H₂O₂ in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C). Incubation times required for completion of the reaction under the experimental conditions employed were determined by computational modeling using Gepasi (84). MtAhpE–SSH was prepared by mixing equimolar concentrations of both H₂O₂ and Na₂S with MtAhpE–SH. Because H₂O₂ reacts several orders of magnitude faster with MtAhpE–SH than with H₂S (8.2 × 10⁴ (50) versus 0.35 M⁻¹ s⁻¹ (57) at pH 7.4 and 25 °C, respectively), MtAhpE–SOH is formed, which in turn reacts with H₂S to form MtAhpE–SSH.

Detection of MtAhpE–SSH by ESI-Q MS

The detection of persulfides was carried out in samples of MtAhpE–SH (10 μM) and Na₂S (10 μM) treated with H₂O₂ (10 μM) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C). At different incubation times, iodoacetamide (40 mM) was added for 30 min at 25 °C. The excess alkylating agent was removed by gel filtration using PD SpinTrap G-25 (GE Healthcare) in 20 mM ammonium bicarbonate buffer (pH 7.4, 25 °C). After this gel filtration step, some samples were further treated with DTT (2 mM) before analysis. All samples were loaded into a C4 column (GraceVydac 214MS5115) for HPLC separation. Mobile phase consisted of 0.1% formic acid in nanopure water (solvent A) and 0.1% formic acid in CH₃CN (solvent B), and elution of the protein was performed with a 10-min linear gradient of solvent B (5–50%) followed by an additional 10 min at 50% solvent B at 100 μl/min. An ESI-triple quadrupole mass
Formation and reactivity of persulfide in MtAhpE

spectrometer (QTrap4500, ABSciex) was employed for detection. The spectrometer was set in Q1 positive mode in the 500–2000 m/z range with a scan rate of 200 Da/s and Q1 resolution in UNIT. Parameters used were as follows: IS, 5000; TEM, 300; DP, 120; EP, 10; CUR, 20; GS1, 30; GS2, 20. Data acquisition was done using Analyst 1.6.2 (ABSciex) and PeakView 2.2 (ABSciex) software was used for data analysis and deconvolution of all spectra.

Kinetics of \( \text{H}_2\text{S} \) reaction with MtAhpE–SOH

The kinetics of the reaction of MtAhpE–SOH with \( \text{H}_2\text{S} \) was determined by two competition approaches, using a SX20 Applied Photophysics stopped-flow spectrophotometer and either absorbance or fluorescence detectors. In the competition of \( \text{H}_2\text{S} \) and TNB for MtAhpE–SOH, the latter (1.4 \( \mu \text{M} \)) was mixed with solutions of \( \text{Na}_2\text{S} \) (0–50 \( \mu \text{M} \)) and TNB (30 \( \mu \text{M} \)) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C), and the absorbance was recorded at 412 nm (\( \epsilon_{412} \) = 14,150 M\(^{-1}\) cm\(^{-1}\)) (82). The exponential rate constants were obtained from the best fits to exponential plus straight line functions. The second-order rate constant of the reaction between \( \text{H}_2\text{S} \) and MtAhpE–SOH was obtained from the slope of the plot of \( k_{\text{obs}} \) versus \( \text{Na}_2\text{S} \) concentration.

The competition of \( \text{H}_2\text{O}_2 \) and \( \text{H}_2\text{S} \) for MtAhpE–SOH was studied following total fluorescence emission (\( \lambda_{\text{ex}} \) = 295 nm), taking advantage of the changes in the protein intrinsic fluorescence that occur during MtAhpE–SH oxidation to MtAhpE–SOH and over-oxidation to sulfenic acid (MtAhpE–SO₂H) as described before (50). MtAhpE–SH (1 \( \mu \text{M} \)) was mixed with solutions of \( \text{Na}_2\text{S} \) (0 or 1 \( \mu \text{M} \)) and \( \text{H}_2\text{O}_2 \) (10, 100, 250 \( \mu \text{M} \)) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C). Results were fitted and modeled using DynaFit (85) to determine the rate constant for the reaction of \( \text{H}_2\text{S} \) toward MtAhpE–SOH. The initial concentration of the reagents was considered as well as the previously reported rate constant for the oxidation of MtAhpE–SH to MtAhpE–SOH and that of MtAhpE–SOH to MtAhpE–SO₂H (50).

Reactivity of MtAhpE–SSH toward DTDPy

MtAhpE–SOH (1.3 \( \mu \text{M} \)) was prepared as described above in a syringe of the SX20 Applied Photophysics stopped-flow spectrophotometer. Then, \( \text{Na}_2\text{S} \) (1.3 \( \mu \text{M} \)) was added to initiate the formation of MtAhpE–SSH. This solution was mixed at different aging times with DTDPy (100–700 \( \mu \text{M} \)), and the absorbance at 324 nm (\( \epsilon_{324} \) = 21,400 M\(^{-1}\) cm\(^{-1}\)) was recorded. Data were fitted to double-exponential functions to obtain the \( k_{\text{obs}} \) amplitudes of the phases. The rate constant of the reaction of MtAhpE–SSH with DTDPy was calculated from the slope of the plot of the observed rate constants for the fast phase versus DTDPy concentration obtained for mixtures of MtAhpE–SH, \( \text{H}_2\text{O}_2 \) and \( \text{Na}_2\text{S} \) aged for at least 900 s.

Reactivity of MtAhpE–SSH toward peroxynitrite and \( \text{H}_2\text{O}_2 \)

The decay of persulfide (12 \( \mu \text{M} \)) in the absence or presence of MtAhpE–SH, MtAhpE–SOH or MtAhpE–SSH (15 \( \mu \text{M} \)) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C) was followed at 310 nm using an SX20 Applied Photophysics stopped-flow spectrophotometer. The reduction of \( \text{H}_2\text{O}_2 \) by the different forms of MtAhpE was followed using the ferrous oxidation–xylene orange method (FOX assay) (51, 86). Briefly, MtAhpE–SH, MtAhpE–SOH or MtAhpE–SSH (45 \( \mu \text{M} \)) were mixed with \( \text{H}_2\text{O}_2 \) (100 \( \mu \text{M} \)) in 0.05 M phosphate buffer (pH 7.4, 25 °C). Aliquots (100 \( \mu \text{l} \)) were taken at different times, mixed with 900 \( \mu \text{l} \) of the FOX reagent, and further incubated for 30 min at room temperature followed by absorbance measurement at 560 nm. The extinction coefficient for \( \text{H}_2\text{O}_2 \) using this assay was determined (\( \epsilon_{560} \) = 51,520 M\(^{-1}\) cm\(^{-1}\)) and was in close agreement with previously reported values (51). The second-order rate constants of the reduction of these oxidants by the different forms of MtAhpE were estimated by initial velocity kinetics.

Catalytic consumption of \( \text{H}_2\text{O}_2 \) and \( \text{H}_2\text{S} \) by MtAhpE

\( \text{H}_2\text{O}_2 \) (125 \( \mu \text{M} \)) and \( \text{Na}_2\text{S} \) (250 \( \mu \text{M} \)) were mixed in the absence or presence of increasing concentrations of MtAhpE–SH (5–29 \( \mu \text{M} \)) in 0.1 M phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C) using sealed vials. Samples were removed using gas-tight syringes and the remaining \( \text{H}_2\text{S} \) was determined by the methylene blue method (87). Alternatively, the same concentrations of \( \text{H}_2\text{O}_2 \) and \( \text{Na}_2\text{S} \) used in the previous experiment were mixed in the absence or presence of MtAhpE–SH (2.9 \( \mu \text{M} \)) in 0.05 M phosphate buffer (pH 7.4, 25 °C), and the remaining \( \text{H}_2\text{O}_2 \) was determined by the FOX assay as described above.

Molecular dynamics of MtAhpE–SH and MtAhpE–SSH

Classical MD of the MtAhpE dimer were performed for the thiolate form of MtAhpE–SH (MtAhpE–S⁻) and for the persulfide form (MtAhpE–SS⁻). For the MtAhpE–S⁻ MD, the recently reviewed crystal structure of MtAhpE (PDB code 4X1U) (88) was used as the starting structure. The persulfide initial model was generated by a modification of the oxidized structure of MtAhpE (PDB code 4X1U) (88) in which the sulfenic acid was in silico transformed to the persulfide form (MtAhpE–SS⁻). Classical parameters for simulating cysteine persulfide were developed using standard protocols (89).

Both initial models were submitted to the same MD protocol as we have previously performed for this and other related enzymes (45, 58, 90). Briefly, the system was solvated with an octahedral box 12 Å in radius with TIP3P water molecules (91). With the exception of cysteine persulfide in the MtAhpE–SS⁻ model, residue parameters correspond to the parm14SB AMBER force field (92). Simulations were performed using periodic boundary conditions with a 10 Å cutoff and a particle mesh Ewald summation method for treating the electrostatic interactions. The hydrogen bond lengths were kept at their equilibrium distance by using the SHAKE algorithm, whereas temperature and pressure were kept constant with a Langevin thermostat and barostat, respectively, as implemented in the AMBER14 program. The system was minimized in 1000 steps (10 with steep gradient and the rest with the conjugate gradient). Then, it was heated from 0 to 300 K for 20 ps at constant pressure with a Berendsen thermostat, and pressure was equilibrated at 1 bar for 5 ps. After these two steps, a 50-ns MD long simulation at a constant temperature (300 K) and a constant volume was performed. An unrestrained 700-ns-long production MD at the NPT ensemble was performed. All dynamics
visualizations and molecular drawings were performed with VMD 1.9.1 (93).

**Transfer of the sulfane sulfur of MtAhpE–SSH to the thiol in SSP4**

MtAhpE–SOH was prepared by incubation of MtAhpE–SH (4 μM) with H2O2 (4 μM) in 0.1 mM phosphate buffer with 0.1 mM DTPA (pH 7.4, 25 °C) during 2 min. MtAhpE–SSH was formed by adding Na2S (4 μM) to sulfenic acid preparations and aging during 15 min. Then, samples were diluted 2-fold in phosphate buffer and mixed with SSP4 (20 μM and 4% DMSO, final concentrations). Fluorescence emission at 515 nm (λex = 482 nm) was recorded in a Varioskan Flash plate reader (Thermo Fisher Scientific). Calibration curves were performed with Na2S2 assuming equimolarity of sulfane sulfur and Na2S2 in reference solutions. The yield of sulfane sulfur transfer to the probe was estimated based on the amplitude of the fit of the fluorescence increase to an exponential function.

**Data processing**

Data were plotted and analyzed using OriginPro 8.0 (OriginLab). Unless specified, results are expressed as the mean ± S.D. of independent experiments.

**Author contributions**—E. C., A. M. R., B. A., and M. T. conceptualization; E. C., A. M. R., A. Z., M. M., R. R., B. A., and M. T. resources; E. C., A. M. R., A. Z., M. M., R. R., B. A., and M. T. formal analysis; E. C., A. M. R., R. R., B. A., and M. T. funding acquisition; E. C., A. M. R., B. A., and M. T. validation; E. C., A. M. R., A. Z., M. M., and M. I. D. A. investigation; E. C., A. M. R., and A. Z. visualization; E. C., A. M. R., A. Z., M. M., B. A., and M. T. writing-original draft; E. C., A. M. R., B. A., and M. T. project administration; E. C., A. M. R., A. Z., M. M., M. I. D. A., R. R., B. A., and M. T. writing-review and editing; B. A. and M. T. supervision.

**References**

1. Abe, K., and Kimura, H. (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 16, 1066–1071 CrossRef Medline
2. Hosoki, R., Matsuki, N., and Kimura, H. (1997) The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem. Biophys. Res. Commun.* 237, 527–531 CrossRef Medline
3. Wang, R. (2012) Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol. Rev.* 92, 791–896 CrossRef Medline
4. Nathan, C., and Shiloh, M. U. (2000) Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8841–8848 CrossRef Medline
5. Shiloh, M. U., and Nathan, C. F. (2000) Reactive nitrogen intermediates and the pathogenesis of *Salmonella* and mycobacteria. *Curr. Opin. Microbiol.* 3, 35–42 CrossRef Medline
6. Alvarez, M. N., Puello, G., Piacenza, L., and Radi, R. (2011) Intraphalangosom peroxynitrite as a macrophage-derived cytotoxin against internalized *Trypanosoma cruzi*: consequences for oxidative killing and role of microbial peroxiredoxins in infectivity. *J. Biol. Chem.* 286, 6627–6640 CrossRef Medline
7. Picenza, L., Triuljio, M., and Radi, R. (2019) Reactive species and pathogen antioxidant networks during phagocytosis. *J. Exp. Med.* 216, 501–516 CrossRef Medline
8. Nambi, S., Long, J. E., Mishra, B. B., Baker, R., Murphy, K. C., Olive, A. J., Nguyen, H. P., Shaffer, S. A., and Sassetti, C. M. (2015) The oxidative stress network of *Mycobacterium tuberculosis* reveals coordination between radical detoxification systems. *Cell Host Microbe* 17, 829–837 CrossRef Medline
9. Cuevaovante, E., Denicola, A., Alvarez, B., and Möller, M. N. (2012) Solubility and permeation of hydrogen sulfide in lipid membranes. *PLoS ONE* 7, e34562 CrossRef Medline
10. Hatzios, S. K., and Bertozzi, C. R. (2011) The regulation of sulfur metabolism in *Mycobacterium tuberculosis*. *PLoS Pathog.* 7, e1002036 CrossRef Medline
11. Bhave, D. P., Muse, W. B., 3rd, and Carroll, K. S. (2007) Drug targets in mycobacterial sulfur metabolism. *Infect. Disord. Drug Targets* 7, 140–158 CrossRef Medline
12. Wheeler, P. R., Coldham, N. G., Keating, L., Gordon, S. V., Wooff, E. E., Parish, T., and Hewinson, R. G. (2005) Functional demonstration of reverse transulfuration in the *Mycobacterium tuberculosis* complex reveals that methionine is the preferred sulfur source for pathogenic mycobacteria. *J. Biol. Chem.* 280, 8069–8078 CrossRef Medline
13. Nszunji, L., Ali, M. K., Wang, X., Huang, X., Yang, W., Duan, X., Yan, S., Li, C., Abdalla, A. E., Jayakumar, P., and Xie, J. (2019) *Mycobacterium tuberculosis* metC (Rv3340) derived hydrogen sulphide conferring bac­teria stress survival. *J. Drug Target* 2019, 1–13 CrossRef Medline
14. Hughes, M. N., Centelles, M. N., and Moore, K. P. (2009) Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. *Free Radic. Biol. Med.* 47, 1346–1353 CrossRef Medline
15. Cuevoante, E., Lange, M., Bonanata, J., Coitoño, E. L., Ferrer-Sueta, G., Filipovic, M. R., and Alvarez, B. (2015) Reaction of hydrogen sulfide with disulfide and sulfenic acid to form the strongly nucleophilic persulfide. *J. Biol. Chem.* 290, 26866–26880 CrossRef Medline
16. Wright, C. M., Christman, G. D., Snellinger, A. M., Johnston, M. V., and Mueller, E. G. (2006) Direct evidence for enzyme persulfide and disulfide intermediates during 4-thiouridine biosynthesis. *Chem. Commun.* 2006, 3104–3106 CrossRef Medline
17. Mueller, E. G. (2006) Trafficking in persulfides: delivering sulfur in bio­synthetic pathways. *Nat. Chem. Biol.* 2, 185–194 CrossRef Medline
18. Ida, T., Sawa, T., Ihara, H., Tsuchiya, Y., Watanabe, Y., Kumagai, Y., Sue-matsu, M., Motohashi, H., Fujii, S., Matsunaga, T., Yamamoto, M., Ono, K., Devarie-Baez, N. O., Xian, M., Fukuto, J. M., and Akaike, T. (2014) Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7606–7611 CrossRef Medline
19. Yadav, P. K., Yamada, K., Chiku, T., Koutmos, M., and Banerjee, R. (2013) Structure and kinetic analysis of H2S production by human mercaptopyrurate sulfurransferase. *J. Biol. Chem.* 288, 2002–2013 CrossRef Medline
20. Jackson, M. R., Melideo, S. L., and Jorns, M. S. (2012) Human sulfide: quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. *Biochemistry* 51, 6804–6815 CrossRef Medline
21. Libiad, M., Melideo, S. L., and Jorns, M. S. (2014) Organization of the human mitochondrial hydrogen sulfide oxidation pathway. *J. Biol. Chem.* 289, 30901–30910 CrossRef Medline
22. Melideo, S. L., Jackson, M. R., and Jorns, M. S. (2014) Biosynthesis of a central intermediate in hydrogen sulfide metabolism by a novel human sulfurransferase and its yeast ortholog. *Biochemistry* 53, 4739–4753 CrossRef Medline
23. Libiad, M., Li, T., Ihara, H., Tsuchiya, Y., Watanabe, Y., Kumagai, Y., Sue-matsu, M., Motohashi, H., Fujii, S., Matsunaga, T., Yamamoto, M., Ono, K., Devarie-Baez, N. O., Xian, M., Fukuto, J. M., and Akaike, T. (2014) Reactive cysteine persulfides and S-polythioliation regulate oxidative stress and redox signaling. *J. Biol. Chem.* 289, 2675–2686 CrossRef Medline
24. Hildebrandt, T. M., and Grishaber, M. K. (2008) Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J.* 275, 3352–3361 CrossRef Medline
25. Tsoohey, J. I. (2011) Sulfur signaling: is the agent sulfide or sulfane? *Anal. Biochem.* 413, 1–7 CrossRef Medline
26. Filipovic, M. R., Zivanovic, J., Alvarez, B., and Banerjee, R. (2018) Chemical biology of H2S signaling through persulfidation. *Chem. Rev.* 118, 1253–1357 CrossRef Medline
Formation and reactivity of persulfide in MtAhpE

27. Francoeleon, N. E., Carrington, S. J., and Fukuto, J. M. (2011) The reaction of H(2)S with oxidized thiol: generation of persulfides and implications to H(2)S biology. Arch. Biochem. Biophys. 516, 146–153 CrossRef Medline

28. Greiner, R., Pálínkás, Z., Básell, K., Becker, D., Antelmann, H., Nagy, P., and Dick, T. P. (2013) Persulfides link H2S to protein thiol oxidation. Antioxid. Redox Signal. 19, 1749–1765 CrossRef Medline

29. Bestetti, S., Medrafo-Fernandez, I., Galli, M., Ghiotti, M., Bienert, G. P., Musco, G., Orsi, A., Rubartelli, A., and Sitia, R. (2018) A persulfidation-based mechanism controls aquaporin-8 conductance. Sci. Adv. 4, eaar5770 CrossRef Medline

30. Artaud, I., and Galardon, E. (2014) A persulfide analogue of the nitrosylated SNAP: formation, characterization and reactivity. ChemBiochem 15, 2361–2364 CrossRef Medline

31. Ubuka, T. (2002) Assay methods and biological roles of labile sulfur in animal tissues. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 781, 227–249 CrossRef Medline

32. Pan, J., and Carroll, K. S. (2013) Persulfide reactivity in the detection of protein S-sulfhydration. ACS Chem. Biol. 8, 1110–1116 CrossRef Medline

33. Zhang, D., Macinkovic, I., Devarie-Baiz, N. O., Pan, J., Park, C., M., Carroll, K. S., Filipovic, M. R., and Xian, M. (2014) Detection of protein S-sulfhydration by a tag-switch technique. Angew. Chem. Int. Ed. Engl. 53, 575–581 CrossRef Medline

34. Nagahara, N., Nirasawa, T., Yoshii, T., and Niimura, Y. (2012) Is novel signal transducer sulfur oxide involved in the redox cycle of persulfide at the catalytic site cysteine in a stable reaction intermediate of mercaptopyruvate sulfurtransferase? Antioxid. Redox Signal. 16, 747–753 CrossRef Medline

35. Bailey, T. S., Zakharov, L. N., and Pluth, M. D. (2014) Understanding hydrogen sulfide storage: probing conditions for sulfide release from hydrosulfides. J. Am. Chem. Soc. 136, 10573–10576 CrossRef Medline

36. Wedmann, R., Onderka, C., Wei, S., Szijártó, I. A., Miljkovic, J. L., Mitrovic, A., Lange, M., Savitsky, S., Yadav, P. K., Torregrossa, R., Harrer, E. G., Harrer, T., Ishii, L., Gollasch, M., Wood, M. E., et al. (2016) Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation. Chem. Sci. 7, 3414–3426 CrossRef Medline

37. Dóka, É., Pader, I., Bíró, A., Johansson, K., Cheng, Q., Ballagó, K., Prigge, D. A., and Trujillo, M. (2015) Oxidizing substrates specificity of Mycobacterium tuberculosis AhpE: kinetic and computational study. Free Radic. Biol. Med. 51, 464–473 CrossRef Medline

38. Hugo, M., Turell, L., Manta, B., Botti, H., Monteiro, G., Netto, L. E., Alvarez, B., Radi, R., and Trujillo, M. (2009) Thiol and sulfenic acid oxidation of AhpE, the one-cysteine peroxiredoxin from Mycobacterium tuberculosis: kinetics, acidity constants, and conformational dynamics. Biochemistry 48, 9416–9426 CrossRef Medline

39. Reyes, A. M., Hugo, M., Trostchansky, A., Capece, L., Radi, R., and Trujillo, M. (2011) Oxidizing substrate specificity of Mycobacterium tuberculosis alkyl hydroperoxide reductase E: kinetics and mechanisms of oxidation and over-oxidation. Free Radic. Biol. Med. 51, 464–473 CrossRef Medline

40. Reyes, A. M., Hugo, M., Trostchansky, A., Capece, L., Radi, R., and Trujillo, M. (2014) Mycothiol/mycobreathindependent reduction of the peroxiredoxin AhpE from Mycobacterium tuberculosis. J. Biol. Chem. 289, 5228–5239 CrossRef Medline

41. Assefa, S. I., Luck, B., and Busch, D. (1998) Kinetics and mechanism of oxidation of hydrogen sulfide by persulfides and persulfides. Biochim. Biophys. Acta 1341, 29–36 CrossRef Medline

42. Portillo-Ledesma, S., Sardi, F., Manta, B., Tourn, M. V., Clippe, A., Knoopos, B., Alvarez, B., Coitoi, L. E., and Ferrer-Sueta, G. (2014) Deconstructing the catalytic efficiency of peroxiredoxin-5 peroxidatic cysteine. Biochemistry 53, 6113–6125 CrossRef Medline

43. Hall, A., Parsonage, D., Poole, L. B., and Karplus, P. A. (2010) Structural evidence that peroxiredoxin catalytic power is based on transition-state stabilization. J. Mol. Biol. 402, 194–209 CrossRef Medline

44. Roque, F., Sardi, F., Manta, B., Tourn, M. V., Clippe, A., Knoopos, B., Alvarez, B., Coitoi, L. E., and Ferrer-Sueta, G. (2014) Deconstructing the catalytic efficiency of peroxiredoxin-5 peroxidatic cysteine. Biochemistry 53, 6113–6125 CrossRef Medline

45. Hall, A., Parsonage, D., Poole, L. B., and Karplus, P. A. (2010) Structural evidence that peroxiredoxin catalytic power is based on transition-state stabilization. J. Mol. Biol. 402, 194–209 CrossRef Medline

46. Portillo-Ledesma, S., Sardi, F., Manta, B., Tourn, M. V., Clippe, A., Knoopos, B., Alvarez, B., Coitoi, L. E., and Ferrer-Sueta, G. (2014) Deconstructing the catalytic efficiency of peroxiredoxin-5 peroxidatic cysteine. Biochemistry 53, 6113–6125 CrossRef Medline
62. Chen, W., Liu, C., Peng, B., Zhao, Y., Pacheco, A., and Xian, M. (2013) New fluorescent probes for sulfane sulfurs and the application in bioimaging. Chem. Sci. 4, 2892–2896 CrossRef Medline

63. Cox, A. G., Winterbourn, C. C., and Hampton, M. B. (2009) Mitochondrial peroxiredoxin involvement in antioxidant defence and redox signalling. Biochem. J. 425, 313–325 CrossRef Medline

64. Trujillo, M., Ferrer-Sueta, G., and Radd, R. (2008) Peroxynitrite detoxification and its biologic implications. Antioxid. Redox Signal. 10, 1607–1620 CrossRef Medline

65. Portillo-Ledesma, S., Randall, L. M., Parsonage, D., Dalla Rizza, J., Karpus, P. A., Poole, L. B., Denicola, A., and Ferrer-Sueta, G. (2018) Differential kinetics of two-cysteine peroxiredoxin disulfide formation reveal a novel model for peroxide sensing. Biochemistry 57, 3416–3424 CrossRef Medline

66. Peshenko, I. V., and Shichi, H. (2001) Oxidation of active center cysteine of bovine 1-Cys peroxiredoxin to the cysteine sulfenic acid form by peroxide and peroxynitrite. Free Radic. Biol. Med. 31, 292–303 CrossRef Medline

67. Loumaye, E., Ferrer-Sueta, G., Alvarez, B., Rees, J. F., Clippe, A., Knoops, B., Radd, R., and Trujillo, M. (2011) Kinetic studies of peroxiredoxin 6 from Arenicola marina: rapid oxidation by hydrogen peroxide and peroxynitrite but lack of reduction by hydrogen sulfide. Arch. Biochem. Biophys. 514, 1–7 CrossRef Medline

68. Wood, Z. A., Poole, L. B., and Karplus, P. A. (2003) Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. Science 300, 650–653 CrossRef Medline

69. Newton, G. L., and Fahey, R. C. (2002) Mycothiol biochemistry. J. Bacteriol. 184, 514–518 CrossRef Medline

70. Chen, W., Liu, C., Peng, B., Zhao, Y., Pacheco, A., and Xian, M. (2013) New fluorescent probes for sulfane sulfurs and the application in bioimaging. Chem. Sci. 4, 2892–2896 CrossRef Medline

71. Cumming, B. M., Lamprecht, D. A., Wells, R. M., Saini, V., Mazorodze, J. H., and Steyn, A. J. C. (2014) Hydrogen peroxide as oxidant and its biological implications. J. Med. Chem. 57, 292–303 CrossRef Medline

72. Pal, V. K., Bandyopadhyay, P., and Singh, A. (2018) Hydrogen sulfide in physiology and pathogenesis of bacteria and viruses. IUBMB Life 70, 393–410 CrossRef Medline

73. Abhishek, S., Saikia, U. N., Gupta, A., Bansal, R., Gupta, V., Singh, N., Laal, S., and Verma, I. (2018) Transcriptional profile of Mycobacterium tuberculosis in an in vitro model of intraocular tuberculosis. Front. Cell. Infect. Microbiol. 8, 330 CrossRef Medline

74. Bonanata, J., Turell, L., Antmann, L., Ferrer-Sueta, G., Botasini, S., Méndez, E., Alvarez, B., and Coitiño, E. L. (2017) The thiol of human serum albumin: acidity, microenvironment and mechanistic insights on its oxidation to sulfenic acid. Free Radic. Biol. Med. 108, 952–962 CrossRef Medline

75. Peskin, A. V., Low, F. M., Paton, L. N., Maghzal, G. J., Hampton, M. B., and Winterbourn, C. C. (2007) The high reactivity of peroxiredoxin 2 with H(2)O(2) is not reflected in its reaction with other oxidants and thiol reagents. J. Biol. Chem. 282, 11885–11892 CrossRef Medline

76. Nelson, D. F., and Kiesow, L. A. (1972) Enthalpy of decomposition of hydrogen peroxide by catalase at 25 °C (with molar extinction coefficients of H(2)O(2) and H(2)O(2) in the UV). Anal. Biochem. 49, 474–478 CrossRef Medline

77. Alvarez, B., Demicheli, V., Durán, R., Trujillo, M., Cerveñansky, C., Freeman, B. A., and Radi, R. (2004) Inactivation of human Cu/Zn superoxide dismutase by peroxynitrite and formation of histidinyl radical. Free Radic. Biol. Med. 37, 813–822 CrossRef Medline

78. Bohle, D. S., Hansert, B., Paulson, S. C., and Smith, B. D. (1994) Biomimetic synthesis of the putative cytoxin peroxynitrite, ONOO−, and its characterization as a tetramethylammonium salt. J. Am. Chem. Soc. 116, 7423–7424 CrossRef Medline

79. Turell, L., Botti, H., Carballal, S., Ferrer-Sueta, G., Souza, J. M., Durán, R., Freeman, B. A., Radi, R., and Alvarez, B. (2008) Reactivity of sulfenic acid in human serum albumin. Biochemistry 47, 358–367 CrossRef Medline

80. Li, S., Peterson, N. A., Kim, M. Y., Kim, C. Y., Hung, L. W., Yu, M., Lekin, T., Segelke, B. W., Lott, J. S., and Baker, E. N. (2005) Crystal structure of AhpE from Mycobacterium tuberculosis, a 1-Cys peroxiredoxin. J. Mol. Biol. 346, 1035–1046 CrossRef Medline

81. Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J. C., Williams, K. L., Appel, R. D., and Hochstrasser, D. F. (1999) Protein identification and analysis tools in the ExPaSy server. Methods Mol. Biol. 112, 531–552 Medline

82. Rienner, C. K., Kada, G., and Gruber, H. J. (2002) Quick measurement of protein sulfhydrols with Ellman’s reagent and with 4,4’-dithiodipyrindine. Anal. Bioanal. Chem. 373, 266–276 CrossRef Medline

83. Grassetti, D. R., and Murray, J. F., Jr. (1967) Determination of sulfhydryl groups with 2,4’- or 4,4’-dithiodipyrindine. Arch. Biochem. Biophys. 119, 41–49 CrossRef Medline

84. Mendes, P. (1993) GEPAASI: a software package for modelling the dynamics, steady states and control of biochemical and other systems. Comput. Appl. Biosci. 9, 563–571 Medline

85. Kuzmic, P. (1996) Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV protease. Anal. Biochem. 237, 260–273 CrossRef Medline

86. Siegel, L. M. (1965) A direct microdetermination for sulfide. Anal. Chem. 37, 813–822 CrossRef Medline

87. Siegel, L. M. (1965) A direct microdetermination for sulfide. Anal. Chem. 37, 813–822 CrossRef Medline

88. van Bergen, J. J., Alonso, M., Palló, A., Nilsson, L., De Proft, F., and Messens, J. (2016) Revisiting sulfur H-bonds in proteins: the example of peroxiredoxin AhpE. Sci. Rep. 6, 30569 CrossRef Medline

89. Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A., and Case, D. A. (2004) Development and testing of a general amber force field. J. Comput. Chem. 25, 1157–1174 CrossRef Medline

90. Reyes, A. M., Vazquez, D. S., Zeida, A., Hugo, M., Pin˜eyro, M. D., De Armas, M. L. Estrin, D., Radi, R., Santos, J., and Trujillo, M. (2016) PrxQ B from Mycobacterium tuberculosis is a monomeric, thioredoxin-dependent and highly efficient fatty acid hydroperoxide reductase. Free Radic. Biol. Med. 101, 249–260 CrossRef Medline

91. Jorgensen, W. L., Chandrasekhar, J., Madura, J. D., Impey, R. W., and Klein, M. L. (1983) Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935 CrossRef Medline

92. Maier, J. A., Martinez, C., Kasavajhala, K., Wickstrom, L., Hauser, K. E., and Simmerling, C. (2015) ff14SB: improving the accuracy of protein side chain and backbone parameters from f99SB. J. Chem. Theory Comput. 11, 3696–3713 CrossRef Medline

93. Humphrey, W., Dalke, A., and Schulten, K. (1996) VMD: visual molecular dynamics. J. Mol. Graph. 14, 33–38, 27–28 CrossRef Medline

94. Nelson, L. J., Knutson, S. T., Soto, L., Klimshir, C., Poole, L. B., and Petrow, J. S. (2011) Analysis of the peroxiredoxin family: using active site structure and sequence information for global classification and residue analysis. Proteins 79, 947–964 CrossRef Medline

95. Kapopoulou, A., Lew, J. M., and Cole, S. T. (2011) The MycoBrowser portal: a comprehensive and manually annotated resource for mycobacterial genomes. Tuberculosis (Edinb) 91, 8–13 CrossRef Medline