Peroxiredoxin 2 is a member of the mammalian peroxiredoxin family of thiol proteins that is important in antioxidant defense and redox signaling. We have examined its reactivity with various biological oxidants, in order to assess its ability to act as a direct physiological target for these species. Human erythrocyte peroxiredoxin 2 was oxidized stoichiometrically to its disulfide-bonded homodimer by hydrogen peroxide, as monitored electrophoretically under nonreducing conditions. The protein was highly susceptible to oxidation by adventitious peroxide, which could be prevented by treating buffers with low concentrations of catalase. However, this did not protect peroxiredoxin 2 against oxidation by added H$_2$O$_2$. Experiments measuring inhibition of dimerization indicated that at pH 7.4 catalase and peroxiredoxin 2 react with hydrogen peroxide at comparable rates. A rate constant of 1.3 × 10$^7$ M$^{-1}$ s$^{-1}$ for the peroxiredoxin reaction was obtained from competition kinetic studies with horseradish peroxidase. This is 100-fold faster than is generally assumed. It is sufficiently high for peroxiredoxin 2 to be a favored cellular target for hydrogen peroxide, even in competition with catalase or glutathione peroxidase. Reactions of ε-butyln and cumene hydroperoxides with peroxiredoxin were also fast, but amino acid chloramines reacted much more slowly. This contrasts with other thiol compounds that react many times faster with chloramines than with hydrogen peroxide. The alkylating agent iodoacetamide also reacted extremely slowly with peroxiredoxin 2. These results demonstrate that peroxiredoxin 2 has a tertiary structure that facilitates reaction of the active site thiol with hydrogen peroxide while restricting its reactivity with other thiol reagents.

Human peroxiredoxin 2 (Prx2)$^3$ and other members of the peroxiredoxin superfamily catalyze the reduction of hydrogen peroxide and organic hydroperoxides through the oxidation and subsequent reduction of cysteine residues (1, 2). The initial step in the catalytic cycle is oxidation of Cys-50 (designated the peroxidatic cysteine, C$_p$) to a sulfenic acid, which reacts with Cys-171 (the resolving cysteine, C$_r$) of another subunit. The result is a disulfide-bonded homodimer of the two subunits linked in a head to tail manner. Physiologically, the catalytic action of Prx2 is understood to involve reduction by thioredoxin, which is recycled by thioredoxin reductase and NADPH (Scheme 1) (3). The disulfide bonds can also be reduced by low molecular weight reductants such as dithiothreitol (DTT).

Peroxiredoxins can function as antioxidants by removing H$_2$O$_2$. They are also proposed to have a signaling role through the "floodgate" mechanism (4) whereby higher concentrations of H$_2$O$_2$ inactivate the protein by overoxidizing C$_p$ to the sulfonic acid (5), thus enabling the peroxide to interact with other cell regulatory pathways. For these mechanisms to operate, the peroxiredoxin must react sufficiently rapidly with H$_2$O$_2$ to compete with other peroxide-metabolizing enzymes, in particular catalase and glutathione peroxidase. Prx2 is one of the predominant cytoplasmic peroxiredoxins in mammalian cells (6). It is relatively abundant, with a concentration of ~0.25 mM estimated for the erythrocyte (7). The ability of Prx2 to break down peroxides catalytically has been demonstrated (6), but absolute rates constants have not been measured. Based on kinetic measurements with microbial 2-Cys peroxiredoxins, rate constants for oxidation of the mammalian proteins by H$_2$O$_2$ are generally assumed to be in the 10$^5$ M$^{-1}$ s$^{-1}$ range (8). In contrast, values for catalases and glutathione peroxidases are closer to 10$^4$ M$^{-1}$ s$^{-1}$. On this basis, even allowing for its cellular concentration being higher, it is debatable whether Prx2 would be able to compete efficiently with the other enzymes (8). Yet there are numerous mammalian cells or whole animal studies where antioxidant or signaling activity of Prx2 has been observed (9–12). Recently, AhpC, a 2-Cys peroxiredoxin from Salmonella typhimurium has been shown to react more rapidly with H$_2$O$_2$ than has been assumed, with a catalytic efficiency of 4 × 10$^7$ M$^{-1}$ s$^{-1}$ (13). Therefore, there is a clear need for more information on the human enzyme.

Our objective was to determine the rate of reaction of human Prx2 with H$_2$O$_2$ and relate this to the reactivity of other peroxides, hypochlorous acid, and a range of chloramines. In undertaking this investigation, initial experiments were thwarted by an inability to remove reducing agents from isolated Prx2 and maintain it in a reduced form. Our study has revealed extreme sensitivity to H$_2$O$_2$ and other peroxides but surprisingly low reactivity with chloramines and resistance to alkylation of the
Peroxiredoxin 2 Reacts with $H_2O_2$ Preferentially

![Diagram of Peroxiredoxin 2 reaction with $H_2O_2$](image)

Catalytic thiols by iodoacetamide. We describe these findings along with kinetic studies that show that Prx2 reacts with $H_2O_2$ with a rate constant comparable with that of catalase.

**EXPERIMENTAL PROCEDURES**

Sodium hypochlorite, hydrogen peroxide, and ethanolamine were obtained from Bio-Rad. Polyvinylidene difluoride membranes and enhanced chemiluminescence plus Western blotting detection systems were from Amersham Biosciences. Rabbit polyclonal anti-Prx2 antibody was obtained from LabFrontier (Seoul, Korea). Goat anti-rabbit antibody conjugated with horseradish peroxidase and other chemicals were from Sigma.

Peroxiredoxin 2 was purified from human erythrocytes obtained from freshly drawn heparinized blood, based on the method of Lim et al. (14). Erythrocytes were washed three times with phosphate-buffered saline, pH 7.4, (PBS) and lysed in 50 mM Tris-HCl buffer, pH 7.6, containing 0.5 mM EDTA and 1 mM DTT. After centrifugation the lysate was treated with 30% ammonium sulfate, and the supernatant was dialyzed against lysis buffer and loaded onto a HiPrep 16/10 DEAE FF column (Amersham Biosciences). The column was washed with this buffer to remove most of the hemoglobin, and bound proteins were eluted with a linear 0–250 mM NaCl gradient. Aliquots of the fractions were analyzed by electrophoresis and Western blotting for the presence of Prx2, and those containing relatively pure Prx2 were pooled. Based on silver-stained SDS gels, we estimate that isolated Prx2 was >90% pure. Purified protein gave a single monomer peak at an $m/z$ ratio of 21,805 by electrospray mass spectrometry and had no detectable catalase activity. Thiold concentration was measured by reduction of NADPH and had no catalase activity. Thiold concentration was measured by reduction of trospray mass spectrometry and had no catalase activity.

The rate constant for reaction of Prx2 with $H_2O_2$ was determined by competition with horseradish peroxidase (HRP). The peroxidase is converted by $H_2O_2$ to compound I, which in the absence of a reducing substrate remains stable under these conditions (17). Spectra were recorded using a NanoDrop spectrophotometer (Biolab Nanodrop Technologies, Wilmington, DE). The concentration of HRP was determined by measuring $A_{403}$ ($e_{403} = 1.02 \times 10^5$ M$^{-1}$ cm$^{-1}$), and the extent of conversion to compound I was monitored at 403 nm.

Mass spectrometry was performed using an LCQ$^\text{TM}$ DECA XP plus ion trap instrument (ThermoFinnigan, San Jose, CA). The desalted protein sample was diluted 1:1 with acetonitrile containing 0.1% formic acid. Detergent was removed from samples containing SDS by extraction with chloroform/methanol/water (18). The sample was directly infused using a Hamilton syringe at a flow rate of 5 $\mu$l/min. A full scan for the mass range 100–2000 $m/z$ was monitored. Data were collected for 1 min before deconvolution using BioworksBrowser 3.1 SR1 (ThermoFinnigan).

**RESULTS**

Prx2 Oxidation by $H_2O_2$ Present in Buffers—To study the reactivity of Prx2, it is imperative to maintain its free thiol groups in the absence of reducing agents. We initially attempted to reduce Prx2 with DTT and remove the DTT using spin columns. Immediate analysis by nonreducing SDS-PAGE showed that most protein in the eluate was present as the disulfide-bonded dimer (Fig. 1A and B, lane 1), which was reduced to the monomer by addition of DTT (lane 2). The dimer was formed even if NEM was placed in the collection tube to alkylate the thiol groups of the protein (not shown), suggesting that oxidation was very rapid and could occur dur-
Peroxiredoxin 2 Reacts with H$_2$O$_2$ Preferentially

![FIGURE 3](image)

**FIGURE 3. Protection of peroxiredoxin 2 oxidation by high concentrations of bovine liver catalase (A) or human erythrocyte catalase (B).** Reduced Prx2 (0.15 mg/ml prepared as for Fig. 2B) was treated with 5 mM H$_2$O$_2$ in the presence of indicated amounts of catalase. NEM (40 mM) was added after 5 min, followed by nonreducing SDS-PAGE and Coomassie staining.

Consistent with the available oxidant being limited. As it was impractical to use large amounts of protein for each of the following experiments, separation of Prx2 from DTT was performed in the presence of catalase for the remainder of the study. This yielded predominantly reduced monomer, although in some experiments dimer was present in the starting material.

**Prx2 Competes Efficiently with Catalase for H$_2$O$_2$—**The ability of Prx2 to be oxidized in the presence of catalase (Fig. 2B) implies that its reaction rate with H$_2$O$_2$ may be greater than is generally considered. Competition reactions performed with catalase established that this is indeed the case. As shown in Fig. 3A, very high concentrations of bovine liver catalase were required to inhibit Prx2 oxidation by H$_2$O$_2$. Analysis of band intensities showed that oxidation of Prx2 (0.15 mg/ml; 6 mM) was 50% protected with $\sim$0.4 mg/ml (7 mM heme) catalase. Thus, both proteins reacted with H$_2$O$_2$ at comparable rates. In an analogous competition experiment with catalase from human erythrocytes (Fig. 3B), 50% protection was observed with $\sim$0.05 mg/ml (0.8 mM heme), indicating that Prx2 was about 8-fold less active than the human catalase preparation. These IC$_{50}$ values are consistent with the 6-fold difference measured in specific activity of the catalase preparations (see “Experimental Procedures”), and with the second-order rate constants of $6 \times 10^6$ and $3 \times 10^7$ M$^{-1}$ s$^{-1}$ for reaction of H$_2$O$_2$ with ferric bovine and human catalase, respectively (20). These data imply a second-order rate constant for the reaction of Prx2 with H$_2$O$_2$ of $\sim$6 $\times 10^6$ M$^{-1}$ s$^{-1}$.

**Determination of the Rate Constant for Reaction of Prx2 with H$_2$O$_2$ by Competition with Horseradish Peroxidase—**To obtain a more accurate value for the Prx2 rate constant, we used the procedure of Ogusucu et al. (17) and measured the ability of Prx2 to compete with HRP for H$_2$O$_2$ and inhibit conversion of the native enzyme to compound I. Compound I formation measured spectrally at 403 nm was progressively inhibited by increasing concentrations of Prx2 (Fig. 4). The fractional inhibition ($I$) was calculated for each Prx2 concentration, and values of $I/([1 - I])_\text{HRP}[HRP]$ s$^{-1}$ were plotted against $[\text{Prx2}]_i$ (Fig. 4, inset). Taking a value of $1.7 \times 10^7$ M$^{-1}$ s$^{-1}$ for $k_\text{HRP}$, the slope of this line gave a second-order rate constant for the reaction of Prx2 with H$_2$O$_2$ of $1.3 \times 10^7$ M$^{-1}$ s$^{-1}$.

**Reactivity of Prx2 with Other Oxidants—**Prx2 also reacted rapidly with organic hydroperoxides. Competition with HRP could not be used to obtain rates for these peroxides, but like H$_2$O$_2$, low concentrations of t-butyl hydroperoxide and cumene hydroperoxide caused rapid Prx2 dimerization within 20 s (Fig. 5).

Inclusion of a small amount of catalase in the equilibration buffer for the spin column resulted in recovery of the majority of the Prx2 in the monomeric state (Fig. 2B, *1st lane*). Protection was seen whether or not DTPA or desferrioxamine was added to inhibit metal-catalyzed oxidation. These results could be explained if Prx2 were highly sensitive to H$_2$O$_2$ and there was a transition was seen whether or not DTPA or desferrioxamine was added to inhibit metal-catalyzed oxidation. These results could be explained if Prx2 were highly sensitive to H$_2$O$_2$ and there was a transition...
We also investigated Prx2 reactivity with HOCl and chloramines. HOCl is generated by the neutrophil enzyme myeloperoxidase and reacts with amine groups to form chloramines.

Thiols are highly favored targets for both HOCl and chloramines, and generally react much faster with these oxidants than with H$_2$O$_2$. For example, rate constants for different chloramines reacting with reduced glutathione at pH 7.4 range from 100 to 700 m$^{-1}$ s$^{-1}$ compared with 0.9 m$^{-1}$ s$^{-1}$ for H$_2$O$_2$ (15, 19, 21). However, the reaction of Prx2 with glycine chloramine was slow. Dimerization was scarcely detectable at 5 min with 40 mM chloramine (not shown), and with 100 mM it required ~20 min for completion (Fig. 6A). An estimate based on half the protein being oxidized by 100 mM glycine chloramine in 5–10 min gives a rate constant of ~10 m$^{-1}$ s$^{-1}$, more than 20 times less than that for the equivalent reaction of reduced glutathione.

The reactivity of Prx2 with other chloramines of varying size and charge was also examined (Fig. 6B). Histamine chloramine was more effective than glycine chloramine, whereas taurine chloramine was less effective. This is a similar order of reactivity to that seen with reduced glutathione and other low molecular weight thiols, but the absolute rates with Prx2 are all slower. The reactivity of monochloramine was strikingly higher than the other chloramines, with 3 mM causing complete dimerization within 5 min (Fig. 6B). The chloramines tested, apart from monochloramine, are charged. To probe the role of charge versus size on reactivity, we used the chloramine of ethanolamine, which is similar in size to glycine chloramine but uncharged. It oxidized Prx2 more rapidly than glycine chloramine but was not nearly as reactive as monochloramine (Fig. 6B).

Prx2 was rapidly oxidized by HOCl (Fig. 6C), although a greater excess was required than with H$_2$O$_2$ (compare with Fig. 2). HOCl shows little discrimination between different thiols (15), and this may be a result of all three thiols of Prx2 being oxidized equally.

**Alkylation of Prx2**—Even though Prx2 is highly susceptible to oxidation by H$_2$O$_2$, we found it surprisingly resistant to alkylation. Prx2 was treated with various concentrations of iodoacetamide (IAM) at pH 7.4 in the presence of low concentrations of peroxide. After 20 s, 17 mM NEM was added. Samples were then separated by nonreducing SDS-PAGE and silver-stained. There was no change in the oxidation state of Prx2 incubated without oxidant under these conditions. A time course for 100 mM glycine chloramine; B, Prx2 oxidation by chloramines derived from ammonia (NH$_3$; monochloramine), glycine (Gly), taurine (Tau), histamine (His), or ethanolamine, all incubated for 5 min; C, concentration dependence for HOCl.

**FIGURE 4.** Determination of second-order rate constant for reaction of Prx2 with H$_2$O$_2$ by competition with horseradish peroxidase. Solutions containing 10 mM HRP and Prx2 in the 7 to 28 mM range in 100 mM potassium phosphate buffer, pH 7.4, were treated with 8 mM H$_2$O$_2$. Spectra were recorded after 2 min using a NanoDrop photometer with a 1-mm light path length, and the change in light path, and the change in A$_{403}$ was determined for each Prx2 concentration. Inset, determination of rate constant (see text for relationship).

**FIGURE 5.** Prx2 oxidation by peroxides. Aliquots containing 5 mM Prx2 (prepared as for Fig. 2B) were mixed with 4 mM peroxide. After 20 s, 17 mM NEM was added, and samples were separated by SDS-PAGE with silver staining. Lane 1, no peroxide; lane 2, H$_2$O$_2$; lane 3, t-butyl hydroperoxide; lane 4, cumene hydroperoxide.

**FIGURE 6.** Concentration and time dependence of oxidation of Prx2 by chloramines and HOCl. Prx2 (5 mM, prepared as for Fig. 2B) was treated with each chloramine or HOCl at the indicated concentration, and at the indicated time, 7 mM NEM was added. Samples were then separated by nonreducing SDS-PAGE and silver-stained. There was no change in the oxidation state of Prx2 incubated without oxidant under these conditions. A, time course for 100 mM glycine chloramine; B, Prx2 oxidation by chloramines derived from ammonia (NH$_3$; monochloramine), glycine (Gly), taurine (Tau), histamine (His), or ethanolamine, all incubated for 5 min; C, concentration dependence for HOCl.
Both NEM and IAM, by forming covalent adducts with the thiol groups, should prevent oxidation of Prx2 to the dimer. This was investigated in the presence of DTT but no catalase by measuring oxidation because of endogenous peroxide in the buffer. There was no dimerization when either NEM (Fig. 8A) or IAM (Fig. 8B) was added at a lower concentration than the DTT. However, with a slight excess of each, dimerization was evident. It became inhibited with high concentrations of NEM but not IAM. These results can be explained by both agents reacting faster with DTT than the Prx2 thiols. Once the DTT is blocked, dimer accumulates because of oxidation by adventitious H$_2$O$_2$. High concentrations of NEM can prevent this by outcompeting the H$_2$O$_2$, but IAM reacts too slowly to stop oxidation. The IAM reaction involves thiolate anions and is very slow with DTT, which is largely nonionized at pH 7.4. Therefore, it is remarkable that Prx2 is even less reactive.

Detergent Changes the Reactivity of Prx2—The presence of SDS during removal of DTT by gel filtration made Prx2 more resistant to oxidation by H$_2$O$_2$, with minimal dimerization occurring at pH 7.4 in the absence of catalase (Fig. 9A). In contrast, SDS added to the reaction mixture made Prx2 more reactive with glycine chloramine (Fig. 9B) and with IAM, which gave only one mass peak corresponding to three alkylated residues (Fig. 7F).

DISCUSSION

We have established that human Prx2 reacts much more rapidly with H$_2$O$_2$ than has been generally appreciated, with a rate
Peroxiredoxin 2 Reacts with \( \text{H}_2\text{O}_2 \) Preferentially

FIGURE 9. SDS changes the reactivity of Prx2 with \( \text{H}_2\text{O}_2 \) and glycine chloramine. A, 5 \( \mu \text{M} \) Prx2 reduced by DTT and passed through a spin column pre-equilibrated with PBS with or without 1% SDS. B, Prx2 (5 \( \mu \text{M} \)) treated with 100 \( \mu \text{M} \) glycine chloramine (GNC) in the presence or absence of 1% SDS.

constant comparable with that of catalase and glutathione peroxidases. This is particularly apparent in Fig. 3 where protein staining shows that more catalase protein than Prx2 was required to inhibit Prx2 dimerization. Furthermore, the presence of small amounts of catalase in Prx2 preparations did not change the stoichiometry of its reaction with \( \text{H}_2\text{O}_2 \). The catalase inhibition data enabled us to estimate that rate constants for the reaction of both proteins with \( \text{H}_2\text{O}_2 \) were comparable. This was quantified by measuring the inhibition of horseradish peroxidase oxidation to compound I, which gave a value of 1.3 \( \times \) 10^{-9} M^{-1} s^{-1}.

The discovery of the peroxiredoxins, while adding a new player to the team of hydrogen peroxide-removing enzymes (1), has created some confusion in defining their role in antioxidant defense. The problem has been that the estimated rate constant for the reaction of Prx2 (and related family members) with \( \text{H}_2\text{O}_2 \) was so much less than that of catalase and glutathione peroxidase that it seemed the peroxiredoxins were “not efficient enough to compete with catalase or the seleno-peroxidases” (8). The 100-fold higher value obtained here using two independent methods overcomes this dilemma. It means Prx2 reacts with \( \text{H}_2\text{O}_2 \) as fast as catalase, and because it is a relatively abundant cellular protein, should be a very effective \( \text{H}_2\text{O}_2 \) scavenger or sensor. This has been demonstrated directly for the erythrocyte (22).

The reactivity of Prx2 may have been underestimated previously because measurements were based on the rates of NADPH oxidation (6) and therefore reflected the regeneration by the thioredoxin reductase system rather than oxidation by \( \text{H}_2\text{O}_2 \). The rate constant we obtained for Prx2, although higher than anticipated, is comparable with the 1–2 \( \times \) 10^{-7} M^{-1} s^{-1} measured for thioredoxin peroxidases I and II from \textit{Saccharomyces cerevisiae} (17) and the 4 \( \times \) 10^{-7} M^{-1} s^{-1} for the bacterial peroxiredoxin AhpC (13). These values were also obtained by direct kinetic measurements rather than from NADPH oxidation.

We found that \( \text{t-} \)butyl hydroperoxide and cumene hydroperoxide oxidized Prx2 rapidly. The rates of disappearance in Fig. 5 indicate that they react at least 1000 times faster than the amino acid chloramines, but it is not possible to determine whether they are as reactive as \( \text{H}_2\text{O}_2 \). The reaction with HOCl was also fast. However, the excess of HOCl required for complete Prx2 dimerization, together with the known high reactivity of HOCl, suggests that this was not a specific reaction with the active site thiol. Apart from monochloramine, all the chloramines tested reacted slowly with Prx2. This is in striking contrast to other thiol compounds, which have been shown to react 100–1000 times faster with chloramines than with \( \text{H}_2\text{O}_2 \) (15, 19, 23). Furthermore, chloramines react with thiolate anions (15) and are therefore expected to favor low pK thiols. The peroxidatic cysteine (Cp) of Prx2 is reputed to have a low pKc (24), and the pH profile for oxidation by \( \text{H}_2\text{O}_2 \) in Fig. 2 suggests a value of 5–6. Yet our estimated rate constants for the amino acid chloramines reacting with Prx2 are substantially lower than for GSH, which has a pKc of 8.8.

The reason for the anomalous behavior of Prx2 was explored by comparing different chloramines. The observed order of reactivity monochloramine > histamine > glycine > taurine chloramine is similar to that for low molecular weight thiols (21). However, the latter do not show the large difference between monochloramine and the other oxidants seen with Prx2. For example, the rate constant for the reaction of monochloramine with 5-thio-2-nitrobenzoic acid (1.4 \( \times \) 10^{-4} M^{-1} s^{-1})^4 is only 1.5 times higher than for histamine chloramine (21). On the assumption that Cp is ionized, these results suggest that its accessibility to the amino acid chloramines may be restricted. The much higher reactivity with monochloramine could reflect its smaller size, although the faster reaction of uncharged ethanolamine chloramine compared with glycine chloramine suggests that charge may also contribute.

Prx2 also reacted slowly with alkylating agents. Treatment with 50 mM iodoacetamide for 8 min at pH 7.4 left most of the protein undervatized. Alkylation increased with increasing iodoacetamide concentration (or time; data not shown) but was never complete, and under most conditions only one of the three thiols became modified. The observed dimerization of Prx2 when excess iodoacetamide was added to solutions containing DTT can be explained by preferential alkylation of the DTT so that it was no longer available to reverse Prx2 oxidation by adventitious \( \text{H}_2\text{O}_2 \). This low reactivity compared with DTT pK_{SMT} 9.2 is not expected of a low pK thiol. Reaction with NEM was faster, with most of the protein alkylated at two sites and substantial signal representing all three thiols labeled. However, the reaction was slow enough for Prx2 to dimerize in solutions containing DTT when NEM was added at a slight excess over the DTT.

Our results indicate that reduced Prx2 is exceptionally reactive with \( \text{H}_2\text{O}_2 \) and other peroxides but shows very low reactivity with other thiol oxidants and alkylating agents. A possible explanation is that the reactive site is configured to favor peroxides over other substrates. If so, changing the tertiary structure of the protein should alter its reactivity. In agreement with this proposition, adding SDS substantially increased the reactivity of Prx2 with both glycine chloramine and iodoacetamide while making it more resistant to oxidation by \( \text{H}_2\text{O}_2 \). Therefore, it would appear that the Prx2 tertiary structure provides a specific environment that makes Cp highly reactive with \( \text{H}_2\text{O}_2 \) while at the same time shielding it from reaction with other oxidants and thiol reagents.

4 A. V. Peskin and C. C. Winterbourn, unpublished data.
The high reactivity of H$_2$O$_2$ with Prx2 (and other 2-Cys peroxiredoxins (6)) is, we believe, unprecedented for a thiol protein. Glutathione peroxidases with active site cysteines have 3 orders of magnitude less reactivity than their selenocysteine counterparts (25), whereas Prx2 has a similar reactivity to the selenoenzymes. The features of the active site environment that confer this reactivity are not yet clear. The C$_p$ thiol group is understood to be ionized because of the presence of a proximal Arg residue (24). However, ionization alone is insufficient to confer such high reactivity. Reduced Prx2 exists as a noncovalent decamer (24) and forms a disulfide bond between C$_p$ and C$_R$ on opposing chains when oxidized. It is apparent from the crystal structure that the distance between the two sulfur atoms is too great for this bond to form without structural reorganization involving movement of both Cys residues from clefts in the protein (13). This constraint slows down the dimerization involving movement of both Cys residues from clefts in the protein (13). This constraint slows down the dimerization involving movement of both Cys residues from clefts in the protein (13). The fraction of oxidized protein became less at higher Prx2 concentrations or low pH, but to generate reduced protein in good yield others may also find it necessary to pre-equilibrate spin columns with buffer containing sufficient catalase to remove endogenous H$_2$O$_2$. The low reactivity of the Prx2 active site thiois with alkylating agents also has practical implications. For example, proteomic investigation of thiol redox state involves labeling reduced thiol proteins, usually with an iodoacetamide or maleimide derivative (32, 33). Derivatization with iodoacetamide, especially at neutral pH, is unlikely to be complete, and oxidation during the reaction with both reagents is possible.

We have shown that Prx2 is sufficiently reactive to act directly as a scavenger for H$_2$O$_2$. Its efficiency as a catalyst of peroxide removal should therefore be dictated by the rate at which it is recycled by the thioredoxin/thioredoxin reductase system. Paradoxically, faster recycling also favors inactivation because of overoxidation. This is apparent in Jurkat cells where the dimer does not accumulate and overoxidation occurs (22). In other cells, such as erythrocytes, recycling is slow; overoxidation is not seen, and the dimer accumulates under oxidative stress (22). However, reversible oxidation of peroxiredoxins may not only be an antioxidant activity. The sensitivity and selectivity of Prx2 for low amounts of H$_2$O$_2$, plus the ability to regulate reduction through the thioredoxin cycle, make it well suited to transducing redox signals initiated by H$_2$O$_2$. The conformational change associated with oxidation could affect interactions with other proteins in signaling pathways, or as is the case with yeast peroxiredoxin Gpx3 (34), oxidizing equivalents could be transferred to other regulatory proteins. Potential mechanisms for signal transduction through reversible peroxiredoxin oxidation in mammalian cells warrant further investigation.

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