Accelerated Publication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 5, Issue of February 4, pp. 3125–3128, 2005
Printed in U.S.A.

Reduction of Cysteine Sulfinic Acid by Sulfiredoxin Is Specific to 2-Cys Peroxiredoxins*

Received for publication, October 20, 2004,
and in revised form, December 6, 2004
Published, JBC Papers in Press, December 8, 2004,
DOI 10.1074/jbc.C400496200

Hyun Ae Woo‡§¶, Woojin Jeong‡¶‖, Tong-Shin Chang‡¶‖, Kwang Joo Park‡, Sung Jun Park‡, Jeong Soo Yang‡‡, and Sue Goo Rhee‡§§**

From the ‡Laboratory of Cell Signaling and ‡‡Laboratory of Biophysical Chemistry, NHLBI, National Institutes of Health, Bethesda, Maryland 20892 and the §Center for Cell Signaling Research and Division of Molecular Life Sciences, Ewha Womans University, Seoul 120-750, Korea

Cysteine residues of certain peroxiredoxins (Prxs) undergo reversible oxidation to sulfinic acid (Cys-SO_2H) and the reduction reaction is catalyzed by sulfiredoxin (Srx). Specific Cys residues of various other proteins are also oxidized to sulfinic acid, suggesting that formation of Cys-SO_2H might be a novel posttranslational modification that contributes to regulation of protein function. To examine the susceptibility of sulfinic forms of proteins to reduction by Srx, we prepared such forms of all six mammalian Prx isoforms and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Purified sulfiredoxin reduced the sulfinic forms of the four 2-Cys members (Prx I to Prx IV) of the Prx family *in vitro*, but it did not affect those of Prx V, Prx VI, or GAPDH. Furthermore, Srx bound specifically to the four 2-Cys Prxs *in vitro* and in cells. Sulfinic forms of Prx I and Prx II, but not of Prx VI or GAPDH, present in H_2O_2-treated A549 cells were gradually reduced after removal of H_2O_2; overexpression of Srx increased the rate of the reduction of Prx I and Prx II but did not induce that of Prx VI or GAPDH. These results suggest that reduction of Cys-SO_2H by Srx is specific to 2-Cys Prx isoforms. For proteins such as Prx VI and GAPDH, sulfinic acid formation might be an irreversible process that causes protein damage.

The sulfur atom of cysteine is able to assume several different oxidation states: the −2 state in the sulfhydryl group (–SH), the −1 state in disulfide (–S–S–), the 0 state in sulfenic acid (–SOH), the +2 state in sulfinic acid (–SO_2H), and the +4 state in sulfonic acid (–SO_3H). Reversible oxidation of cysteine to disulfide or sulfenic acid, both of which are readily reduced by thiols such as glutathione (GSH)[1] and thioredoxin (Trx), is an important type of posttranslational modification that contributes to the regulation of protein function. Sulfinic and sulfonic oxidation states are also found in proteins. However, given that sulfinic and sulfonic acids were found not to be reduced by biological thiols under physiological conditions (1), the hyperoxidation reactions that give rise to these moieties were thought to be irreversible and to occur only under conditions of extreme oxidative stress or during protein isolation. The cysteine sulfinic acid produced during the catalytic cycle of peroxiredoxins (Prxs) was nevertheless recently found to be reducible in cells (2–6).

Members of the Prx family of peroxidases are present in organisms from all kingdoms (7–9). All Prx enzymes contain a conserved Cys residue in the NH_2-terminal portion of the molecule, and most contain an additional conserved Cys in the COOH-terminal region. Prx enzymes exist as homodimers, with the two monomers arranged in a head-to-tail manner, and the dimers are able to aggregate further to form decamers (9). Mammalian cells express six isoforms of Prx (Prx I to VI), which are classified into three subgroups (2-Cys, atypical 2-Cys, and 1-Cys) on the basis of the number and position of Cys residues that participate in catalysis (8). Prx I to Prx IV belong to the 2-Cys Prx subgroup, Prx V to the atypical 2-Cys subgroup, and Prx VI to the 1-Cys subgroup. Prx I to Prx IV thus possess the conserved NH_2-terminal Cys (N-Cys) and a COOH-terminal Cys (C-Cys) that are separated by 121 amino acids. During catalysis, peroxides oxidize N-Cys–SH to sulfenic acid, which then reacts with C-Cys–SH of the other subunit to form an intermolecular disulfide. This disulfide is subsequently reduced specifically by Trx. The N-Cys–SH is also the site of oxidation by peroxides in Prx V and Prx VI, neither of which contains a C-Cys. The resulting sulfenic acid of Prx V forms an intramolecular disulfide with a Cys–SH that is separated from N-Cys by 104 amino acids, and the disulfide is reduced by Trx. In the case of Prx VI, the sulfenic acid does not form a disulfide because of the unavailability of another Cys–SH nearby; it can be reduced by nonphysiological thiols such as dithiothreitol (DTT) but not by Trx or GSH.

Studies with 2-Cys Prxs, mainly Prx I, have indicated that the N-Cys–SOH intermediate is occasionally further oxidized to sulfinic acid before it is able to form a disulfide with C-Cys–SH, resulting in inactivation of peroxidase activity (10, 11). This oxidation to sulfinic acid was recently found to be a reversible step (2–4), with the back reaction being catalyzed by each of two ATP-dependent reductases designated sulfiredoxin (Srx) and sestrin (5, 6). The N-Cys–SH of Prx V or Prx VI also undergoes hyperoxidation to sulfinic acid (4). Furthermore, oxidation of Cys to sulfinic acid does not appear to be restricted to Prx enzymes. Critical Cys residues of many other proteins, including α_1-antitrypsin (12), carbonic anhydrase III (13), glyc-

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ These authors contributed equally to this work.
‖ Present address: Center for Cell Signaling Research and Division of Molecular Life Sciences, Ewha Womans University, 11-Daehyun Dong, Seodaemoon-gu, Seoul 120-750, Korea.
** On leave from the Department of Pulmonary and Critical Care Medicine, Ajou University School of Medicine, Suwon 442–749, Korea.
§§ To whom correspondence should be addressed at: Bldg. 50, Rm. 3523, South Dr., MSC 8015, Bethesda, MD 20892. Tel.: 301-496-9646; Fax: 301-480-0357; E-mail: sgrhee@nih.gov.

[1] The abbreviations used are: GSH, glutathione; Trx, thioredoxin; Prx, peroxiredoxin; DTT, dithiothreitol; Srx, sulfiredoxin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HA, hemagglutinin epitope; GST, glutathione *S*-transferase; ESI-MS, electrospray ionization–mass spectrometry; MALDI-TOF, matrix-assisted laser desorption-ionization–time-of-flight; HBSS, Hanks' balanced salt solution.

eraldehyde-3-phosphate dehydrogenase (GAPDH) (14), protein-tyrosine phosphatase 1B (15), and metalloproteinases (16), are also oxidized to sulfinic acid. Indeed, 1.4% of the Cys residues of soluble proteins in rat liver were detected as sulfinic acid, whereas sulfonic acid was not detected (17). The cycle of thiol to sulfinic acid might thus represent a redox switch by which the function of a wide variety of proteins is regulated in response to a change in intracellular redox status. The operation of such a thiol-sulfinic switch would appear to require the existence of sulfinic reductases of broad specificity. We have examined the ability of Srx to catalyze the reduction of various sulfinic proteins, and we now show that Srx appears to act specifically in reduction of the sulfinic acid groups of 2-Cys Prx proteins.

EXPERIMENTAL PROCEDURES

Materials—Preparation of recombinant human Prx I, III, IV, V, and VI (18–21), recombinant rat Srx (22), and recombinant human Trx1 (18) was described previously. Rabbit antisera specific for rat Srx (22), for sulfinylated 2-Cys Prxs (3), for Prx isoform (18–21) or for human Trx1 (23), have also been described. Mouse monoclonal antibodies to β-actin, to the hemagglutinin epitope (HA), to GAPDH, or to glutathione *S*-transferase (GST) were obtained from Abcam, Santa Cruz Biotechnology, Chemicon International, and Santa Cruz Biotechnology, respectively. Rabbit muscle GAPDH was from Sigma. Prx II was purified from outdated human erythrocytes as described (24).

Preparation of Sulfinic Proteins—Human 2-Cys Prxs (Prx I to IV) were oxidized in the presence of H_2O_2 (1 mM), human Trx1, and DTT as described previously for Prx I (2, 3). A similar procedure was followed for the preparation of sulfinic forms of Prx V with the exception that the concentration of H_2O_2 was increased to 3 mM for Prx V. Recombinant human Prx VI (250 μg) or rabbit muscle GAPDH (250 μg) was incubated for 10 min at 30 °C in a 250-μl reaction mixture containing 4 mM H_2O_2 and 50 mM Tris-HCl (pH 7.5). The sulfinic state of the oxidized proteins was verified by electrospray ionization-mass spectrometry (ESI-MS) as described (2, 3).

Generation of Antibodies Specific for Sulfinic Proteins—Three peptides, AFTPGCSKTH, DFTPVCTTEL, and KIISNASCTTN, which correspond to the oxidation-sensitive sites of mammalian Prx V and Prx VI, and mammalian GAPDH, respectively, were oxidized and used to generate rabbit antibodies specific for the sulfinylated proteins as described previously (3).

Construction of Expression Vectors—Complementary DNA encoding human Srx was cloned into the BamHI and NotI sites of pEBG-*Srf*I (kindly provided by Y. Liu, NIA, National Institutes of Health) or the XbaI and BamHI sites of pCGN (kindly provided by W. Herr, Cold Spring Harbor Laboratory) for the expression of a GST-Srx fusion protein or HA-tagged Srx, respectively. Cells were transfected with these vectors with the use of FuGENE 6 (Roche Applied Science).

RESULTS

Srx Reduces the Cys Sulfinic Acid of 2-Cys Prx Enzymes but Not That of Atypical 2-Cys Prx, 1-Cys Prx, or GAPDH in Vitro—Sulfinic forms of the six human Prx isozymes (Prx I to VI) were prepared, and their sulfinic oxidation state was verified by ESI-MS (data not shown). They were then incubated with Srx in the presence of $MgCl_2$ and ATP, and the resulting assay mixtures were subjected to two-dimensional PAGE followed by immunoblot analysis (Fig. 1). Sulfinic Prxs are detected at a more acidic position than are the corresponding reduced proteins because of the presence of the negatively charged sulfinic group. Although the rates of reduction differed, Srx gradually regenerated the reduced forms of the four 2-Cys Prxs. Srx failed to reduce the sulfinic forms of Prx V and Prx VI, however. Similar results were obtained when the regeneration of the reduced enzymes was monitored on the basis of recovery of Trx-dependent peroxidase activity (data not shown). To confirm the different reactivities of Prxs with Srx, we incubated a mixture of the sufinic forms of Prx I, Prx III, and Prx VI with Srx. The two-dimensional analysis of the reaction mixture confirmed that Prx I and Prx III, but not Prx VI, were substrates of Srx (data not shown).

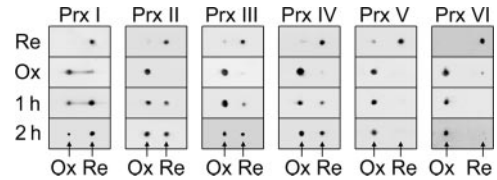

FIG. 1. **Reduction of sulfinic Prx enzymes by Srx *in vitro*.** Sulfinic human Prxs (5 μM) were incubated at 30 °C in a 50-μl reaction mixture containing 1 μM rat Srx, 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM creatine phosphate, creatine phosphokinase (25 milliunits/ml), 1 mM $MgCl_2$, 1 mM ATP, and 5 mM GSH. A portion (15 μl) of the mixture was removed after incubation for 0, 1, or 2 h (*Ox, 1 h, 2 h*) and was subjected to 2D PAGE followed by immunoblot analysis with antibodies specific for the corresponding Prx isozyme. Similar amounts of reduced Prxs (*Re*) were also subjected to the same analysis. The positions of sulfinic (*Ox*) and reduced (*Re*) Prxs are indicated.

We also prepared sulfinic forms of rabbit muscle GAPDH. GAPDH contains four Cys residues, among which the catalytic site Cys^{149}–SH, which is activated by the proton-extracting residue His^{176}, is sensitive to oxidation. Incorporation of two oxygen atoms into Tsa1 and GAPDH during oxidation was detected by ESI-MS. Specific oxidation of Cys^{149} of GAPDH was revealed by collision-induced dissociation-tandem mass spectrometry of tryptic peptides (data not shown). We have previously developed a procedure to monitor the reduction of sulfinic 2-Cys Prx enzymes by immunoblot analysis (after one-dimensional PAGE) with antibodies (anti-2-Cys Prx-SO_2) that specifically recognize both sufinic and sulfonic forms of these proteins. We also produced anti-Prx V-SO_2, anti-Prx VI-SO_2, and anti-GAPDH-SO_2 antibodies. The specificity of these antibodies was apparent from the observation that they detected oxidized (sulfinic) forms but not nonoxidized forms of the corresponding target proteins in immunoblot analysis (Fig. 2). The reduction of the sulfinic forms of Prx I, Prx V, Prx VI, and GAPDH was examined by immunoblot analysis with the corresponding antibodies specific for the hyperoxidized state (Fig. 2). Consistent with the results obtained by two-dimensional analysis, the immunoblot intensity of the Prx I band detected by anti-2-Cys Prx-SO_2 gradually decreased with time of incubation with Srx, whereas that of the band detected with antibodies to Prx I remained unchanged (Fig. 2). In contrast, the band intensities of oxidized Prx V, Prx VI, and GAPDH revealed by the corresponding antibodies to the hyperoxidized state remained unchanged during incubation with Srx. These data indicated that the reduction reaction catalyzed by mammalian Srx is specific to mammalian 2-Cys Prx enzymes.

Srx Binds to 2-Cys Prx Enzymes but Not to Atypical 2-Cys or 1-Cys Prxs—We expressed a GST fusion protein of human Srx (GST-Srx) in HeLa cells and precipitated the fusion protein from the lysates of H_2O_2-treated cells with GSH-agarose beads. Bead-bound proteins were fractionated by SDS-PAGE, and major bands corresponding to proteins that coprecipitated with GST-Srx were excised from the gel, subjected to in-gel digestion with trypsin, and identified by matrix-assisted laser desorption-ionization-time-of-flight (MALDI-TOF) mass spectrometry (data not shown). All major bands, with the exception of that corresponding to a 50-kDa protein, were identified as Prx IV, GST-Mu, Prx III, Prx I, and Prx II (Fig. 3*A*). The 50-kDa band was found to be associated with GSH-agarose beads even when GST-Srx was not expressed in HeLa cells (data not shown). HeLa cells express all six mammalian Prx enzymes; the abundance of Prx I, Prx II, and Prx VI is in the range of 3–7 μg/mg of soluble protein and that of Prx III and Prx V is in the range of 0.3–0.5 μg/mg (8). However, only 2-Cys Prxs, not Prx V nor Prx VI, were found associated with GST-Srx.

Human Srx contains a single Cys residue at position 99 that is essential for reductase activity and has been proposed to

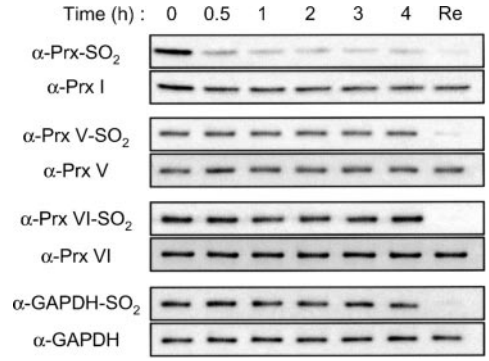

FIG. 2. **Substrate specificity of mammalian Srx for sulfinylated proteins.** Sulfinic forms of Prx I, Prx V, Prx VI, or GAPDH (5 μM concentration of each) were incubated for the indicated times in a reaction mixture containing 1 μM rat Srx, 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM creatine phosphate, creatine phosphokinase (25 milliunits/ml), 1 mM $MgCl_2$, 1 mM ATP, and 5 mM GSH. The oxidation state of each protein was monitored by SDS-PAGE followed by immunoblot analysis with antibodies specific for the sulfinic forms of 2-Cys Prxs, Prx V, Prx VI, or GAPDH, respectively. The blots were reprobed with antibodies to Prx I, Prx V, Prx VI, and GAPDH, respectively. Reduced forms (*Re*) of the proteins were similarly analyzed.

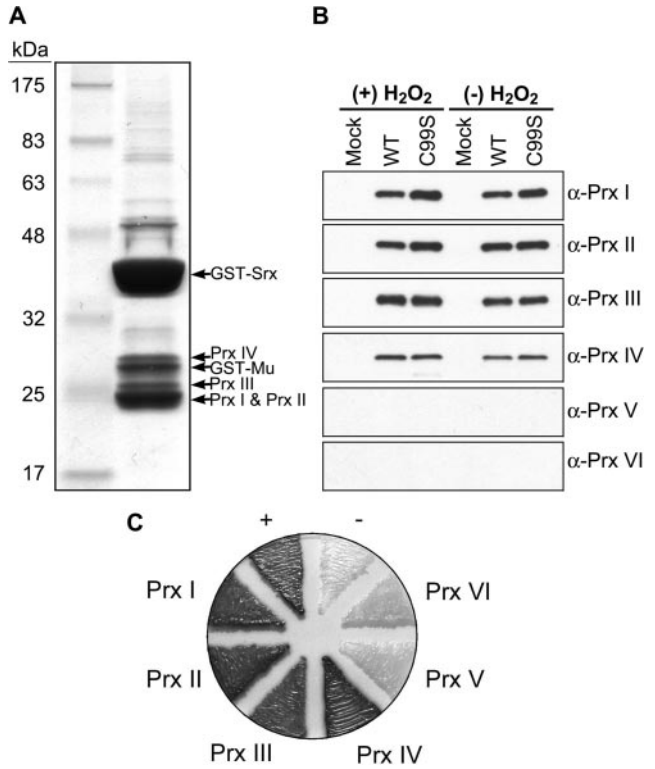

FIG. 3. **Identification of proteins that interact physically with Srx.** *A*, HeLa cells grown in 100-mm dishes were transfected with an expression vector for GST-Srx and cultured for 24 h before treatment for 10 min with 100 μM H_2O_2 in 10 ml of Hanks' balanced salt solution (HBSS). The cells were then washed with HBSS and disrupted in ice-cold lysis buffer (20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 150 mM NaCl, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, leupeptin (10 μg/ml), aprotinin (10 μg/ml)). After centrifugation, the lysates were incubated with GSH-Sepharose resin for 30 min at room temperature. The resin was then isolated by centrifugation and washed three times with lysis buffer, after which bead-bound proteins were fractionated by SDS-PAGE and stained with Coomassie Brilliant Blue R-250. Proteins identified by MALDI-TOF mass spectrometry are indicated (*right lane*); molecular size standards are also shown (*left lane*). *B*, HeLa cells grown in 60-mm dishes were transfected with expression vectors for GST (*Mock*), GST-Srx (*WT*), or GST-Srx (*C99S*) and cultured for 24 h before incubation for 10 min with 4 ml of HBSS in the absence or presence of 100 μM H_2O_2. Proteins precipitated with GSH-Sepharose as described for *A* were subjected to immunoblot analysis with antibodies to (α-) each of the six mammalian isoforms of Prx. *C*, yeast strain EGY48 was sequentially transformed with the p8op *lacZ* reporter plasmid, a pLexA bait plasmid for human Srx, and a pB42AD prey plasmid for Prx I to Prx VI. Colonies resulting from each transformation were assayed by the X-Gal whole-plate method as described (35). The combination of pLexA/p53 and pB42AD/T (Clontech) was used as a positive control (+), resulting in the formation of dark blue colonies, and the combination of pLexA/Srx and pB42AD was used as a negative control (−).

form a thiosulfinate with the sulfinic moiety of Prx during the catalytic cycle (5). To test whether the Srx-Prx interaction indeed involves Cys^{99}, we constructed a mutant (C99S) of human Srx in which Cys^{99} is replaced by Ser. HeLa cells transfected with expression vectors for GST-Srx, GST-Srx(C99S), or GST alone were treated with H_2O_2, after which GST or the GST fusion proteins were precipitated from cell lysates. Immunoblot analysis of the precipitates with antibodies specific for each of the six mammalian Prx isoforms revealed that GST-hSrx interacted with Prx I, Prx II, Prx III, and Prx IV but not with Prx V or Prx VI (Fig. 3*B*). Mutation of Cys^{99} of Srx did not affect its association with the 2-Cys Prxs, indicating that the association does not involve the formation of either a disulfide or a thiosulfinate linkage between Srx and Prx. Furthermore, Srx appeared to interact with oxidized (sulfinic) and nonoxidized 2-Cys Prxs to similar extents, given that omission of H_2O_2 treatment of cells did not affect the association.

We also examined the interaction between Srx and Prx with the yeast two-hybrid system. Yeast cells transformed with a bait plasmid for human Srx and prey plasmids for Prx I, Prx II, Prx III, or Prx IV grew on selective medium and the colonies stained blue for β-galactosidase activity, whereas those transformed with the Srx bait plasmid and prey plasmids for Prx V or Prx VI grew poorly on selective medium (Fig. 3*C*). These results thus provided further support for the notion that Srx interacts only with 2-Cys Prxs.

Overexpression of Srx Promotes the Reduction of Sulfinic Forms of 2-Cys Prxs but Not That of Atypical 2-Cys Prx, 1-Cys Prx, or GAPDH—To study the specificity of Srx in cells, we transfected A549 human lung epithelial cells with an expression vector for HA-tagged human Srx or the corresponding empty vector (Fig. 4). We have previously shown that attachment of GST to or deletion of 16 amino acid residues from the NH_2 terminus of human and rat Srx did not affect its reductase activity measured *in vitro* (22). The transfected cells were exposed to 1 mM H_2O_2 for 10 min to induce protein sulfinylation and were then incubated for various times in the absence of H_2O_2 but in the presence of cycloheximide. Cell lysates were then subjected to immunoblot analysis with anti-2-Cys Prx-SO_2, anti-Prx VI-SO_2, and anti-GAPDH-SO_2 (Fig. 4). The band recognized by anti-2-Cys Prx-SO_2 was broad or partially separated because the molecular sizes of Prx I, Prx II, and Prx III are similar. A gradual reduction of sulfinic 2-Cys Prxs was apparent in the control cells, and this rate was greatly increased in the cells expressing HA-Srx. Treatment of cells with H_2O_2 induced the sulfinylation of Prx VI and GAPDH; however, reduction of the sulfinic forms of Prx VI and GAPDH was not apparent either in control cells or in cells expressing HA-Srx. Prx IV and Prx V were not analyzed in these experiments because of their low concentrations in A549 cells. These results thus indicated that reduction by Srx is specific for 2-Cys Prx enzymes in cells.

DISCUSSION

Our results indicate that Srx specifically binds and reduces the sulfinic forms of 2-Cys Prx isoforms but not those of other proteins exemplified by atypical 2-Cys Prx, 1-Cys Prx, and GAPDH. The binding requires neither the sulfinic oxidation state of the substrate protein nor the active site Cys of Srx. Srx was first identified and characterized in yeast as a sulfinyl re-

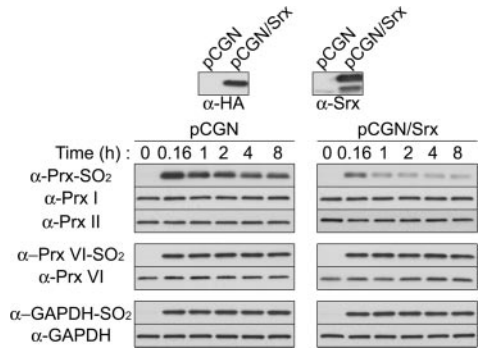

FIG. 4. **Effect of Srx overexpression on the reduction of sulfinic forms of 2-Cys Prxs, Prx VI, and GAPDH in A549 cells.** Cells were subjected to transient transfection by electroporation with the Nucleofector instrument (Amaxa Biosystems) for 36 h with an expression vector for HA-tagged human Srx or with the corresponding empty vector (*pCGN*), after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to HA and to Srx (*upper panels*). The transfected cells were also exposed to 1 mM H_2O_2 for 10 min (0.16 h), washed with HBSS, and incubated for various times in F-12K medium (Invitrogen) supplemented with 10% fetal bovine serum and cycloheximide (10 μg/ml). Cell lysates (20 μg) were then subjected to immunoblot analysis with antibodies specific for the sulfinic forms of 2-Cys Prxs, Prx VI, or GAPDH as well as with antibodies to either Prx I, Prx II, Prx VI, or GAPDH. Times shown refer to the total times of incubation, including the 10-min exposure to H_2O_2.

ductase for the oxidized 2-Cys Prx (5). Human 2-Cys Prx isoforms share 60–80% sequence identity with each other, whereas the sequence identity between 2-Cys Prxs and either atypical 2-Cys Prx or 1-Cys Prx is ~10 and 30%, respectively (8).

Hydrogen peroxide is produced as a result of normal cellular functions such as respiration as well as in response to exposure to radiation, light, and redox-active drugs. Although H_2O_2 itself is not especially reactive, it can be converted to the highly reactive hydroxyl radical. All aerobic cells are therefore equipped with H_2O_2-removing enzymes, which include catalase, glutathione peroxidases, and Prxs. Stimulation of various cell surface receptors also induces the transient production of H_2O_2 (25), and cytosolic Prxs are implicated in the removal of such H_2O_2 (19, 26, 27). Hydrogen peroxide produced in response to receptor stimulation appears to participate in receptor signaling by targeting various molecules, including protein-tyrosine phosphatases and PTEN; oxidation of the essential Cys residues of these proteins and their consequent inactivation by H_2O_2 has thus been demonstrated in various stimulated cell types (25, 28–31).

The facts that prokaryotic orthologs of 2-Cys Prxs are insensitive to oxidative inactivation and that prokaryotes contain neither sulfiredoxin nor sestrin have suggested that 2-Cys Prxs in prokaryotes function only as antioxidants, whereas mammalian 2-Cys Prxs serve as both antioxidants and signal modulators (32). The sulfinylation-dependent inactivation of 2-Cys Prxs was therefore proposed to be the result of structural features acquired during evolution to accommodate the intracellular messenger function of H_2O_2. This function of H_2O_2 likely requires that its concentration increase rapidly above a certain threshold, and this requirement is likely met through protection of the generated H_2O_2 molecules from destruction by Prxs, which are present in high concentrations in the cytosol to remove the low levels of H_2O_2 produced as a result of normal cellular metabolism and oxidative insults. Protection of the signaling function of H_2O_2 is probably transiently provided by the built-in mechanism of inactivation of the two cytosolic 2-Cys Prx enzymes, Prx I and Prx II, mediated by H_2O_2.

Whereas Srx is restricted to the cytosol (22), Prx III, Prx IV, and Prx V are localized predominantly to other compartments (8). Prx III is a mitochondrial protein synthesized with a mitochondrial targeting sequence, Prx IV is posttranslationally processed in the endoplasmic reticulum and secreted into the extracellular space, and Prx V is present in peroxisomes and mitochondria. Although Srx is able to reduce sulfinic Prx III *in vitro*, it remains unclear whether the reduction of Prx III in cells is actually mediated by Srx. It is possible that a sestrin isoform is responsible for this reduction. The substrate specificity and subcellular distribution of sestrins are not known.

Prx VI is also a cytosolic protein (8), but the sulfinic form of Prx VI is neither a substrate of Srx nor reduced in cells. Sulfinic GAPDH was also not reduced either by Srx *in vitro* or in cells. On the basis of these observations, we propose that, in contrast to the reversible phosphorylation of proteins, not all sulfinic proteins are susceptible to reduction in cells. At present, only the 2-Cys Prx isoforms have been shown to cycle between thiol and sulfinic acid states. For certain proteins such as Prx VI and GAPDH, sulfinic acid formation might be an irreversible process that causes protein damage.

Acknowledgments—We thank Y. Liu for pEBG-*Srf*I and W. Herr for pCGN.

REFERENCES

1. Jacob, C., Holme, A. L., and Fry, F. H. (2004) *Org. Biomol. Chem.* **2,** 1953–1956
2. Woo, H. A., Chae, H. Z., Hwang, S. C., Yang, K. S., Kang, S. W., Kim, K., and Rhee, S. G. (2003) *Science* **300,** 653–656
3. Woo, H. A., Kang, S. W., Kim, H. K., Yang, K. S., Chae, H. Z., and Rhee, S. G. (2003) *J. Biol. Chem.* **278,** 47361–47364
4. Chevallet, M., Wagner, E., Luche, S., van Dorsselaer, A., Leize-Wagner, E., and Rabilloud, T. (2003) *J. Biol. Chem.* **278,** 37146–37153
5. Biteau, B., Labarre, J., and Toledano, M. B. (2003) *Nature* **425,** 980–984
6. Budanov, A. V., Sablina, A. A., Feinstein, E., Koonin, E. V., and Chumakov, P. M. (2004) *Science* **304,** 596–600
7. Chae, H. Z., Robison, K., Poole, L. B., Church, G., Storz, G., and Rhee, S. G. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7017–7021
8. Rhee, S. G., Kang, S. W., Chang, T. S., Jeong, W., and Kim, K. (2001) *IUBMB Life* **52,** 35–41
9. Hofmann, B., Hecht, H. J., and Flohe, L. (2002) *Biol. Chem.* **383,** 347–364
10. Chae, H. Z., Kim, I. H., Kim, K., and Rhee, S. G. (1993) *J. Biol. Chem.* **268,** 16815–16821
11. Yang, K. S., Kang, S. W., Woo, H. A., Hwang, S. C., Chae, H. Z., Kim, K., and Rhee, S. G. (2002) *J. Biol. Chem.* **277,** 38029–38036
12. Griffiths, S. W., King, J., and Cooney, C. L. (2002) *J. Biol. Chem.* **277,** 25486–25492
13. Mallis, R. J., Hamann, M. J., Zhao, W., Zhang, T., Hendrich, S., and Thomas, J. A. (2002) *Biol. Chem.* **383,** 649–662
14. Souza, J. M., and Radi, R. (1998) *Arch. Biochem. Biophys.* **360,** 187–194
15. Wang, Q., Dube, D., Friesen, R. W., LeRiche, T. G., Bateman, K. P., Trimble, L., Sanghara, J., Pollex, R., Ramachandran, C., Gresser, M. J., and Huang, Z. (2004) *Biochemistry* **43,** 4294–4303
16. Fu, X., Kassim, S. Y., Parks, W. C., and Heinecke, J. W. (2001) *J. Biol. Chem.* **276,** 41279–41287
17. Hamann, M., Zhang, T., Hendrich, S., and Thomas, J. A. (2002) *Methods Enzymol.* **348,** 146–156
18. Kang, S. W., Baines, I. C., and Rhee, S. G. (1998) *J. Biol. Chem.* **273,** 6303–6311
19. Kang, S. W., Chae, H. Z., Seo, M. S., Kim, K., Baines, I. C., and Rhee, S. G. (1998) *J. Biol. Chem.* **273,** 6297–6302
20. Seo, M. S., Kang, S. W., Kim, K., Baines, I. C., Lee, T. H., and Rhee, S. G. (2000) *J. Biol. Chem.* **275,** 20346–20354
21. Jin, D. Y., Chae, H. Z., Rhee, S. G., and Jeang, K. T. (1997) *J. Biol. Chem.* **272,** 30952–30961
22. Chang, T. S., Jeong, W., Woo, H. A., Lee, S. M., Park, S., and Rhee, S. G. (2004) *J. Biol. Chem.* **279,** 50994–51001
23. Lee, S. R., Kim, J. R., Kwon, K. S., Yoon, H. W., Levine, R. L., Ginsburg, A., and Rhee, S. G. (1999) *J. Biol. Chem.* **274,** 4722–4734
24. Kristensen, P., Rasmussen, D. E., and Kristensen, B. I. (1999) *Biochem. Biophys. Res. Commun.* **262,** 127–131
25. Rhee, S. G., Bae, Y. S., Lee, S.-R., and Kwon, J. (2000) *Science's STKE* **2000,** pe1
26. Zhang, P., Liu, B., Kang, S. W., Seo, M. S., Rhee, S. G., and Obeid, L. M. (1997) *J. Biol. Chem.* **272,** 30615–30618
27. Kang, S. W., Chang, T. S., Lee, T. H., Kim, E. S., Yu, D. Y., and Rhee, S. G. (2004) *J. Biol. Chem.* **279,** 2535–2543
28. Lee, S. R., Kwon, K. S., Kim, S. R., and Rhee, S. G. (1998) *J. Biol. Chem.* **273,** 15366–15372
29. Lee, S.-R., Yang, K.-S., Kwon, J., Lee, C., Jeong, W., and Rhee, S. G. (2002) *J. Biol. Chem.* **277,** 20336–20342
30. Mahadev, K., Zilbering, A., Zhu, L., and Goldstein, B. J. (2001) *J. Biol. Chem.* **276,** 21938–21942
31. Leslie, N. R., Gray, A., Pass, I., Orchiston, E. A., and Downes, C. P. (2000) *Biochem. J.* **346,** 827–833
32. Wood, Z. A., Poole, L. B., and Karplus, P. A. (2003) *Science* **300,** 650–653

Reduction of Cysteine Sulfinic Acid by Sulfiredoxin Is Specific to 2-Cys Peroxiredoxins

Hyun Ae Woo, Woojin Jeong, Tong-Shin Chang, Kwang Joo Park, Sung Jun Park, Jeong Soo Yang and Sue Goo Rhee

J. Biol. Chem. 2005, 280:3125-3128.
doi: 10.1074/jbc.C400496200 originally published online December 8, 2004
