1-Cys Peroxiredoxin, a Bifunctional Enzyme with Glutathione Peroxidase and Phospholipase A₂ Activities*

Received for publication, June 12, 2000, and in revised form, June 30, 2000
Published, JBC Papers in Press, July 12, 2000, DOI 10.1074/jbc.M005073200

Jin-Wen Chen, Chandra Dodia, Sheldon I. Feinstein, Mahendra K. Jain‡, and Aron B. Fisher§

From the Institute for Environmental Medicine, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania 19104 and the ‡Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716

This report provides definitive evidence that the protein 1-Cys peroxiredoxin is a bifunctional (“moonlighting”) enzyme with two distinct active sites. We have previously shown that human, rat, and bovine lungs contain an acidic Ca²⁺-independent phospholipase A₂ (aiPLA₂). The cDNA encoding aiPLA₂ was found to be identical to that of a non-selenium glutathione peroxidase (NSGPx). Protein expressed using a previously reported *E. coli* construct which has a His-tag and 50 additional amino acids at the NH₂ terminus, did not exhibit aiPLA₂ activity. A new construct which contains the His-tag plus two extra amino acids at the COOH terminus when expressed in *Escherichia coli* generated a protein that hydrolyzed the sn-2 acyl chain of phospholipids at pH 4, and exhibited NSGPx activity with H₂O₂ at pH 8. The expressed 1-Cys peroxiredoxin has identical functional properties to the native lung enzyme: aiPLA₂ activity is inhibited by the serine protease inhibitor, diethyl p-nitrophenyl phosphate, by the tetra-hedral mimic 1-hexadecyl-3-trifluoroethylglycero-sn-2-phosphomethanol (MJ33), and by 1-Cys peroxiredoxin monoclonal antibody (mAb) 8H11 but these agents have no effect on NSGPx activity; NSGPx activity is inhibited by mercaptoascuicinate and by 1-Cys peroxiredoxin mAb 8B3 antibody which have no effect on aiPLA₂ activity. Mutation of Ser32 to Ala abolishes aiPLA₂ activity, yet the NSGPx activity remains unaffected; a Cys47 to Ser mutant is devoid of peroxidase activity but aiPLA₂ activity remains intact. These results suggest that Ser47 in the GDSWG consensus sequence provides the catalytic nucleophile for the hydrolysis activity of aiPLA₂, while Cys47 in the PVCTTE consensus sequence is at the active site for peroxidase activity. The bifunctional catalytic properties of 1-Cys peroxiredoxin are compatible with a simultaneous role for the protein in the regulation of phospholipid turnover as well as in protection against oxidative injury.

Evidence has emerged that a lysosomal-type Ca²⁺-independent phospholipase A₂ with acidic pH optimum (aiPLA₂)¹ (1, 2) and a non-selenium glutathione peroxidase without glutathione S-transferase activity (NSGPx) (3, 4) are the same enzyme, based upon their identical cDNA sequence. Furthermore, based upon the cDNA sequence, this protein belongs to the thioredoxin peroxidase, or peroxiredoxin, family although with only one instead of the usual two conserved cysteine residues and thus has been called 1-Cys peroxiredoxin (5). The nomenclature is further confusing since the enzyme does not utilize thioredoxin (4, 5), but rather functions as a GSH peroxidase (3, 4).

Although the cDNA sequence of aiPLA₂ (1) is identical to that of NSGPxs (4), the presence of both activities up to the present has not been confirmed in the same peptide. Native protein isolated from bovine or rat or rabbit mucosa had peroxidase activity but PLₐ₂ activity was not tested (6, 7). Native protein isolated from rat or bovine lungs demonstrated PLₐ₂ activity although peroxidase activity was not tested (1, 2, 8). Likewise, studies of recombinant 1-Cys peroxiredoxin have reported either peroxidase (3, 4) or PLₐ₂ (1, 2) activity. One study (5) did evaluate both peroxidase and PLₐ₂ activity in recombinant protein with somewhat mixed results: 1) the peroxidase activity of the recombinant protein required diithiothreitol and was not supported by GSH; 2) the purified recombinant protein did not have PLₐ₂ activity; 3) a low level of PLₐ₂ activity was detected in NIH 3T3 cells transfected with the cDNA although the activity was not affected by mutagenesis of the putative active site serine of the protein. Thus, the presence of both activities in the same protein remains unconfirmed.

The present study was designed to resolve the apparently contradictory results in the literature. Using a new construct of the cDNA of human 1-Cys peroxiredoxin, we have provided definitive evidence for both peroxidase and phospholipase activities in recombinant protein and have provided evidence for two distinct active sites.

**EXPERIMENTAL PROCEDURES**

*Materials—The MORPH® plasmid DNA mutagenesis kit was purchased from 5 Prime → 3 Prime, Inc. (Boulder, CO). Restriction enzymes were from New England Biolabs, Inc. (Beverly, MA). Plasmid pET-28c and the His-tag purification kit were from Novagen, Inc. (Madison, WI). BL21(DE3) expression cells and pBlueScript SK(+) vector were from Stratagene (La Jolla, CA). The pET-21b vector was a gift from Dr. Dekbarm Pain of the University of Pennsylvania. Oligonucleotides were synthesized by the DNA synthesis facility of the University of Pennsylvania Cancer Center. T7 mMESSAGE mMACHINE® large scale *in vitro* transcription kit and wheat germ IVT™ kits were from Ambion Inc. (Austin, TX). Tran35S-label was from ICN Pharmaceuticals, Inc. (Irvine, CA). GSH, glutathione reductase, NADPH, isopropyl-β-D-thiogalactopyranoside, diethyl p-nitrophenyl phosphate (DNP), mercaptoascuicinate, and H₂O₂ were purchased from Sigma.

DENP, diethyl p-nitrophenyl phosphate; ML33, 1-hexadecyl-3-trifluoroethylglycero-sn-2-phosphomethanol; PC, phosphatidylycerol; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine; bp, base pair(s); PAGE, polyacrylamide gel electrophoresis.

This paper is available on line at http://www.jbc.org

² This work was supported by National Institutes of Health Grant HL19737. 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 1754 solely to indicate this fact.

§ To whom reprint requests should be addressed: Institute for Environmental Medicine, University of Pennsylvania School of Medicine, 1 John Morgan Bldg., 3620 Hamilton Walk, Philadelphia, PA 19104-6068.
Fig. 1. Plasmid constructs for expression of 1-Cys peroxiredoxin. Panel A, construct 1. A 1653-bp human cDNA including the coding region was cloned into pBluescript SK(+) vector. The coding region is boxed, the start codon is underlined, and the termination codon (TAA) is bold. Arrowhead = T7 promoter. This construct was expressed in a wheat germ in vitro translation system. Panel B, construct 2. A 1044-bp human cDNA including the coding region and a His tag at the NH₂ terminus was cloned into pET-28c. Arrowhead = T7 promoter. The insert and His tag are boxed. DNA coding for the start codon is underlined and the termination codon (TAA) is bold. This construct was expressed in E. coli. Panel C, construct 3. A 672-bp cDNA of the human coding region with His tag at the COOH terminus was cloned into pET-21b. The insert and His tag are boxed. DNA coding for the start codon and the last amino acid codon are underlined, and the termination codon (TGA) is bold. This construct was expressed in E. coli.

Bisbodipy-C₁₂-phosphatidylcholine was from Molecular Probes, Inc. (Eugene, OR). 1-Palmitoyl-2-[9,10-3H]palmitoyl-sn-glycerol-3-phosphocholine ([3H]DPPC) was purchased from NEN Life Science Products (Boston, MA). Unlabeled DPPC, egg phosphatidylcholine (PC), and other lipids were purchased from Avanti Polar Lipids (Alabaster, AL). Nitrocellulose membrane, protein dye-binding assay kit, and Triton X-100 (electrophoretically purified) were purchased from Bio-Rad (Hercules, CA). Complete²⁰ protease inhibitor mixture tablets were from Roche Molecular Biochemicals (Indianapolis, IN). Low-range molecular mass standards for SDS-PAGE were from Bio-Rad or Roche Molecular Biochemicals. Enhanced chemiluminescence kit and x-ray film were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Horseradish peroxidase-conjugated Affinity-Pure rat anti-mouse IgG was from Jackson ImmunoResearch Laboratory Inc. (West Grove, PA). 1-Hexadecyl-3-trifluoroethylglycero-sn-2-phosphoethanol (MJ33) was from Molecular Probes, Inc. Cysteine at position 47 was replaced by serine (C47S), and serine at position 32 was substituted by alanine (S32A). Mutagenesis was performed using the MORPH²¹ plasmid DNA mutagenesis kit supplied by 5 Prime (MORPH²¹ plasmid DNA mutagenesis kit). Constructs 2 and 3 were expressed in Escherichia coli while construct 1 was utilized in a wheat germ expression system.

Site-directed Mutagenesis—Mutations were made using the MORPH²¹ plasmid DNA mutagenesis kit supplied by 5 Prime. Cysteine at position 47 was replaced by serine (C47S), and serine at position 32 was substituted by alanine (S32A). Mutagenesis
DNAs were digested with *Not*I, the reaction was transformed into 200 non-mutagenized methylated target plasmid DNA. After chilling for 5 min, the reaction at 37 °C and incubated for 30 min to destroy the original, and then at room temperature for 30 min. The reaction was started by 1-Cys peroxiredoxin (2 μg) in vitro construct 1 in a wheat germ expression system. The 5′-CTCT-3′ mutations are 9′-CCCCAGTGTcCACCACAGAG-3′ which are located between nucleotides 131–150 and 83 and 105, respectively, in the open reading frame of 1-Cys peroxiredoxin (1). The numbering of oligonucleotides in the sense strand of 1-Cys peroxiredoxin begins at the start codon and the mutated nucleotides are shown in lowercase letters. Target plasmid DNA (0.03 pmol) and phosphorylated mutagenic oligonucleotide (50 ng/μl) were denatured at 100 °C for 5 min, incubated in an ice water bath for 5 min and then at room temperature for 30 min. The reaction was started by adding MORPH® synthesis buffer, T4 DNA polymerase, and T4 DNA ligase, and incubation was for 2 h at 37 °C to synthesize a non-methylated replacement strand and to ligate it into a circular molecule. After stopping the reaction by heating to 85 °C for 15 min, DpnI was added to the reaction at 37 °C and incubated for 30 min to destroy the original, non-mutagenized methylated target plasmid DNA. After chilling for 5 min, the reaction was transformed into 200 μl of competent *E. coli mutS* cells, which were plated after heat shock at 42 °C for 2 min. Colonies were screened through digestion of the corresponding plasmid DNA with the specific enzyme for the designed mutation.

The designed mutations were identified by restriction analysis. The introduction of the S32A mutation creates a *HpaI* restriction site so plasmid DNAs prepared from colonies in the mutagenesis experiment were cut with *HpaI* to identify the mutant plasmid DNA that has an extra band of 387 base pairs. The C47S mutation removes an *ApaLI* site, so plasmid DNAs were digested with *ApaLI* to detect the mutant plasmid DNA lacking one band of 1124 base pairs. Subsequently, each mutant DNA was sequenced to further confirm that the DNA had the expected mutation and that this is the only mutation present. Only confirmed mutant DNA was further processed for in vitro expression.

For construct 1, the mutant plasmids were cleaved with *NarI* and *BamHI* to obtain a 372-bp fragment, and the wild-type plasmid DNA was also cleaved with the same enzymes to get a wild type vector that contained the remainder of the insert. The fragment and the wild type vector were purified by agarose gel electrophoresis and were religated in order to avoid unexpected mutations. The presence of the mutated fragment was confirmed by both DNA restriction analysis and sequencing. Construct 2 was not used for mutation analysis. For construct 3, a fragment of 291 base pairs including the mutation sites for S32A or C47S was obtained by digestion of S32A or C47S 1-Cys peroxiredoxin-pBluescript SK(+) with *EcoRI* and *BamHI*, and was subsequently recloned into 1-Cys peroxiredoxin-pET-21b, which had been digested with the same enzymes, effectively replacing this region with the mutated version. The recloned insert was tested for the correct orientation through digestion with EcoRV. DNA sequencing was used to confirm that only the designed mutation was present in the insert.

**In Vitro Transcription—**cRNA was expressed from the 1-Cys peroxiredoxin-pBluescript SK(+) wild type and mutant clones using the T7 mMESSAGE mMACHINE® *in vitro* transcription kit. The template DNAs were digested with *Not*I to linearize the DNA and provide a transcriptional terminus, then purified by phenol/chloroform extraction and isopropyl alcohol precipitation according to standard procedures. A 20-μl transcription reaction was assembled with transcription buffer, ribonucleotide mixture, linearized template DNA (1 μg), and enzyme mixture including transcriptase, and was incubated at 37 °C for 2 h to reach a maximal yield. The remaining template DNA was then removed by adding 2 units of RNase-free DNase I at 37 °C for 15 min. The reaction was terminated by adding 30 μl of nuclease-free distilled H₂O and 25 μl of 0.7 M LiCl with 75 mM EDTA, and was chilled overnight at −20 °C. The solution was centrifuged at 4 °C for 30 min at 14,000 × g to pellet the RNA. The pellet was washed with 70% ethanol. After drying, cRNA was dissolved in nuclease-free distilled H₂O, and was stored at −70 °C. Meanwhile, cRNA concentration was measured at OD₂₆₀/OD₂₈₀ and was then electrophoresed onto a 1% agarose/formaldehyde gel to evaluate its quality. The transcripts for the wild type, C47S and S32A cDNA sequences are shown in Fig. 2A. Their mobility was near their predicted size of approximately 1750 bp, near the 18 S rRNA of rat lung.

**In Vitro Translation—**The recombinant wild type and mutant proteins prepared with construct 1 were expressed in *vitro* in a wheat germ translation kit from Ambion Inc. Briefly, a 50-μl reaction contained 2.5 μl of 1 M potassium acetate, 2.5 μl of minus leucine Master Mix (1 mM amino acids without leucine, 0.16 mM creatine phosphate), 2.5 μl of minus methionine Master Mix (1 mM amino acids without methionine, 0.16 mM creatine phosphate), 25 μl of wheat germ extract, 1 μg of cRNA, and distilled H₂O. The reaction was incubated at 37 °C for 60 min, and stored at 4 °C for further activity assays. Wheat germ translated protein was quantified by 35Smethionine incorporation. In these experiments, 0.5 μM 35Smethionine (63 Ci/mmol) was present in the translation reaction. Otherwise the method was as described previously (4). After translation, 1-Cys peroxiredoxin represented about 0.05% of the total wheat germ extract protein for both wild type and mutant proteins, indicating that wild type and mutant 1-Cys peroxiredoxin cRNA have comparable translation efficiencies. SDS-PAGE with autoradiography of the translated 35Smethionine-labeled protein showed an apparent molecular mass of 26 kDa for wild type, C47S and S32A, similar to the molecular mass deduced from the open reading frame and of the PL₄₅ enzyme isolated from rat lung (Fig. 2B). The 672-bp coding sequence should generate a polypeptide of 25,035 daltons.

Human recombinant proteins with constructs 2 or 3 were expressed as fusion proteins with a series of six histidine residues in BL21(DE3) cells. These cells produce T7 RNA polymerase and express pET-28c and pET21b inserts efficiently. Human wild type 1-Cys peroxiredoxin in construct 2 was grown in LB broth as described previously (4). Based on the intensity of the protein band on Coomassie Blue-stained 12% SDS-PAGE gel (not shown), wild type 1-Cys peroxiredoxin is about 13% of total protein. The proteins were purified through Ni²⁺ columns as described previously (4). The purity of the expressed proteins after the Ni²⁺ column was about 90% as estimated from SDS-PAGE (Fig. 3A). The purified protein migrated with a molecular mass of about 32 kDa on 12% SDS-PAGE (Fig. 3A) and immunoblot probed with the 1-Cys peroxiredoxin mAb 8H11 (Fig. 3B). The theoretical mass of 1-Cys peroxiredoxin with construct 2 is 31,021 daltons.
Wild type, S32A and C47S 1-Cys peroxiredoxin in construct 3 were expressed in M9 minimal medium instead of LB broth since the latter was found to give a better yield. After 15 min induction with 1 mM isopropyl-β-D-thiogalactopyranoside, 100 μg of rifampicin/ml was added to culture medium containing 50 μg of ampicillin/ml to inhibit the bacterial DNA polymerase and block bacterial protein production while not affecting the T7 polymerase and thus allowing an enrichment of 1-Cys peroxiredoxin. The bacteria were collected by centrifugation and resuspended in binding buffer (5 mM imidazole, 0.5 mM NaCl, 20 mM Tris-HCl, pH 7.9). In order to prevent degradation of the recombinant protein, protease inhibitor mixture tablets were added to the binding buffer (one tablet per 25 ml). The crude cell lysate was tested for the presence of the recombinant protein using Coomassie Brilliant Blue gel staining of 12% SDS-PAGE (not shown). Based on the intensity of the protein band on the gel, the expressed wild type, S32A, and C47S proteins represented about 20% of total protein. The protein was purified using the His tag on Ni²⁺ columns. The purity of the recombinant proteins after the Ni²⁺ column was about 96% as estimated from Coomassie-stained gels. This construct generated a protein with a molecular mass of about 27 kDa on 12% SDS-PAGE (Fig. 3A) and immunoblot probed with the 1-Cys peroxiredoxin mAb 8H11 (Fig. 3B). This construct is predicted to produce a protein of 26,100 daltons.

Activities for aiPLA₂ and NSGPx were measured as described under “Experimental Procedures.” aiPLA₂ activity was assayed using [³H]DPPC in mixed unilamellar vesicles as substrate. NSGPx activity was assayed with H₂O₂ in the presence of GSH, NADPH, and GSH reductase. Wheat germ translated protein with construct 1 was labeled with [³S]methionine in order to calculate specific activity. Constructs 2 and 3 represent the purified proteins following E. coli expression. Results are mean ± range for two independent experiments.

### TABLE I

| NSGPx | aiPLA₂ | NSGPx/aiPLA₂ |
|-------|--------|-------------|
|       | pmol/min/mg protein | pmol/min/mg protein |
| **Construct 1** | 4000 ± 120 | 36.3 ± 0.5 |
| **Construct 2** | 1850 ± 84 | 0.04 ± 0.002 |
| **Construct 3** | 5460 ± 170 | 49.8 ± 4.0 |

* The very high ratio for construct 2 is not meaningful.
crude cell extract was also extremely low (data not shown). Therefore, we generated another construct for expression in E. coli containing only two extra amino acids before the six-histidine residues which were placed at the COOH terminus (construct 3 shown in Fig. 1C).

The protein generated from construct 3 exhibited both aiPLA2 and NSGPx activities (Table I). The NSGPx activity for construct 3 was significantly higher than for construct 2 and somewhat higher than for construct 1. For aiPLA2 activity, construct 3 was slightly higher than construct 2 and markedly higher than construct 2. The activity ratios of NSGPx to aiPLA2 in enzyme generated from constructs 1 and 3 were similar and indicate that the peroxidase activity under the conditions of the assay was 2 orders of magnitude greater than the PLA2 activity. Thus, we identified both NSGPx and aiPLA2 activities in translated protein from both wheat germ (construct 1) and E. coli (construct 3) expression systems. Construct 2 exhibited NSGPx but little PLA2 activity suggesting that PLA2 activity was sensitive to protein conformation and also that the active sites for the two activities are different.

Additional evidence for different active sites was obtained by determining the response of the two catalytic activities to inhibitors (Table II). Mercaptosuccinate, an inhibitor of cysteine- and selenocysteine-mediated reactions, inhibited NSGPx activity by 98% at 20 μM, but had no effect on aiPLA2 activity (Table II). Both DENP, a serine protease inhibitor and MJ33, a transition state phospholipid analogue, inhibited aiPLA2 activity by approximately 80–90% but had no effect on NSGPx activity (Table II). Monoclonal antibodies raised against E. coli-expressed human 1-Cys peroxiredoxin also showed differential effects. mAb 8B3 significantly inhibited NSGPx by 88% but aiPLA2 activity was unaffected whereas mAb 8H11 inhibited aiPLA2 by 80% but had no effect on NSGPx activity (Table II).

Identification of Protein Active Sites—Based on the results with DENP and the presence of a putative “lipase” motif (1), we mutated Ser32 to Ala (S32A) in constructs 1 and 3 to study its role in aiPLA2 activity. The S32A mutation abolished PLA2 activity with both constructs 1 and 3 but did not affect NSGPx activity (Table III). The results obtained with construct 3 are illustrated graphically in Fig. 4 using bisbodipy-C11-PC liposomal substrate to assay aiPLA2 and NADPH fluorescence to assay NSGPx. These results suggest that Ser32 in the GDSWG motif is an active site residue for the phospholipase function of 1-Cys peroxiredoxin.

Since the peroxidase activity is inhibited by mercaptosuccinate (Table II), we mutated Cys47, the only conserved Cys in 1-Cys peroxiredoxin, to Ser (C47S). Previously, mutation of this Cys abolished peroxidase activity (5) although the results of this latter study are clouded by the inability of GSH to function as co-factor with the wild type protein. Mutagenesis of Cys47 totally abolished NSGPx activity while PLA2 activity in the mutant remained at the wild type level (Table III and Fig. 4). These results confirm that the enzyme has a Cys-active site at position 47 for NSGPx activity and provide additional evidence for two distinct active sites for the two enzymatic activities of this protein.

**DISCUSSION**

Although one gene-one protein-one function has been a paradigm of biochemistry, an increasing number of exceptions are being reported (11). The term “moonlighting proteins” has been used to designate proteins that have multiple functions (11). The present results with constructs 1 and 3 clearly show the presence of both glutathione peroxidase and phospholipase activities in the same recombinant protein. The activities of the recombinant human protein using construct 3 (5460 for NSGPx and 50 for aiPLA2 in mol/min/mg protein from Table I) were similar to values for native protein isolated from bovine eye (NSGPx, 5070 nmol/min/mg of protein) (6) and bovine lung (aiPLA2, 65 nmol/min/mg of protein) (8). Activities with construct 1 in wheat germ-translated protein and for NSGPx in construct 2 expressed in E. coli were slightly less. aiPLA2 activity with construct 2 was very low which may have been due to an altered serine hydrolase site, possibly caused by misfolding due to the His tag at the NH2 terminus. An analogous effect might account for the lack of aiPLA2 activity in the E. coli expressed protein of Kang et al. (5).

The above evidence indicates that this protein has two separate activities with apparently two distinct active sites, since only one activity was lost with construct 2. Results with the S32A and C47S mutants provide additional evidence for this observation which is further supported by the differential response of the two activities to the inhibitors mercaptosuccinate (cysteine active) and DENP (serine active). Finally, mAb 8B3 and mAb 8H11 each inhibit only one of the two activities.

Our S32A mutation results show that Ser32 is critical for the hydrolase activity of the enzyme, compatible with the inhibi-

---

**Table II**

| Inhibitors | | % Inhibition |
|---|---|---|
| | aiPLA2 | NSGPx | aiPLA2 | NSGPx |
| Mercaptosuccinate (20 μM) | 7 ± 2 | 98 ± 1 | 2 ± 1.4 | 99 ± 0.02 |
| DENP (0.5 mM) | 77 ± 2 | 2 ± 2 | 88 ± 0.5 | 7 ± 2 |
| MJ33 (3 mol %) | 82 ± 1 | 5 ± 1 | 90 ± 0.2 | 2 ± 1 |
| mAb 8B3 | 7 ± 1 | 88 ± 3 | 4 ± 0.5 | 99 ± 1 |
| mAb 8H11 | 80 ± 3 | 0 ± 0 | 86 ± 0.4 | 6 ± 2 |

**Table III**

| | | NSGPx | aiPLA2 |
| | | Construct 1 | Construct 3 | Construct 1 | Construct 3 |
| | | nmol/min/mg | % | nmol/min/mg | % | nmol/min/mg | % |
| S32A | 3900 ± 260 | 98 | 5240 ± 200 | 96 | 43.4 ± 2.9 | 95 | 48.6 ± 4 | 98 |
| C47S | ND | <0.3 | ND | <0.3 | ND | <0.06 | ND | <0.04 |

* ND, none detected; the lower limit of detection is 10 nmol/min/mg for NSGPx and 0.020 nmol/min/mg for aiPLA2.
tion of PLA₂ activity by DENP. DENP inhibits PLA₂ activity of purified rat and bovine lung enzymes as well (2, 8). Serine is also the active site in the 85-kDa cytosolic Ca²⁺-independent phospholipase A₂ (12), the 44-kDa platelet activating factor hydrolase (13), and the 25-kDa lysophospholipase I (14). GDSWG is a conserved sequence in 1-Cys peroxiredoxin that fits the consensus sequence (GXXG) found in serine hydrolases of diverse substrate specificity, such as proteases and lipases (12–16). We conclude that the GDSWG sequence is part of the catalytic site for aiPLA₂. The locations of other residues that make up the putative catalytic triad, Asp-Ser-His (15), remain to be identified. The results also show that Ser³₂ does not participate in H₂O₂ peroxidase activity compatible with results obtained with the serine protease inhibitor (Table II).

The peroxidase activity of the expressed protein is several orders of magnitude greater than the phospholipase activity (Table I). The enzyme can reduce H₂O₂ and short chain organic, fatty acid, and phospholipid hydroperoxides in the presence of GSH (4). Note that classical cytosolic GSHPx has no activity toward phospholipid hydroperoxides (17) although the selenium-dependent phospholipid hydroliperoxide glutathione peroxidase (PHGPx) does have this activity (18). Despite the similarity of peroxidase function, 1-Cys peroxiredoxin has no significant amino acid homology with GSHPx or PHGPx. Unlike 1-Cys peroxiredoxin, GSHPx and PHGPx are both seleno enzymes that require dietary selenium for their synthesis and activity (19). Glutathione S-transferase is also a non-seleno enzyme that can “repair” oxidized fatty acids by thiol transfer and shows a low level of activity toward phospholipid hydroperoxides but is not a true peroxidase and has no activity toward H₂O₂ (20).

Inhibition of GSHPx activity by mercaptosuccinate, confirming the importance of the cysteine residue, has been reported before for the purified protein from bovine eye (6) and for recombinant human protein (4). The conserved Cys⁴⁷ in 1-Cys peroxiredoxin occurs in a PVCTTE cassette that represents a consensus sequence for peroxidase activity and has been confirmed to be a critical site for removing H₂O₂ in some enzymes (21–23) including 1-Cys peroxiredoxin (5). The rat and bovine sequences show a single Cys at position 47 (2, 4). The human enzyme has a second Cys at position 91 but this is not conserved and its mutation has no effect on the peroxidase activity of the enzyme (5). Cys⁹¹ → Ala mutant human 1-Cys peroxiredoxin has been crystallized as a dimer (24). The x-ray crystal structure study demonstrated the location of the active site cysteine at the bottom of a narrow pocket and indicated that Cys⁷ exists as Cys-SOH (cysteine-sulfenic acid) in the oxidized native 1-Cys peroxiredoxin (24). For wild type enzyme, the reaction mechanism could be partially due to formation of Cys-SOH from reaction between enzyme and H₂O₂ (5). From the kinetic data, it is evident that the mutant enzyme, C47S, is catalytically disabled for peroxidase. Therefore, cysteine in 1-Cys peroxiredoxin performs the role reserved for selenocysteine in GSHPx and PHGPx.

Maximal peroxidase activity was shown to occur between pH 7 and 8, concordant with a cytosolic localization for 1-Cys peroxiredoxin. In contrast to the pH 7–8 optimum for GSHPx activity, aiPLA₂ activity is maximal at pH 4 and essentially non-existent at pH 6 and above. By subcellular fractionation, 1-Cys peroxiredoxin protein has been localized to cytosol and also to lysosomes and lung secretory organelles (8, 25) where the pH is in the appropriate range for aiPLA₂ activity (26). We have provided evidence previously that an enzyme inhibited by MJ33, presumably aiPLA₂, functions in the metabolism of phospholipids in lung surfactant (25, 27). In addition, aiPLA₂ could function synergistically with GSHPx during oxidative stress. In the cytosolic compartment, the enzyme, through GSHPx activity, could directly reduce peroxidized plasma membrane phospholipids. For phospholipid hydroperoxides that might be transferred to the lysosomal compartment, this enzyme could release peroxidized fatty acids from the sn-2 position of phospholipids for their subsequent reduction in the cytoplasm. Thus, 1-Cys peroxiredoxin may function ubiquitously in the repair of oxidized (peroxidized) membranes and could be considered a general enzyme for antioxidant defense.

Acknowledgments—We thank Dr. Debkumar Pain for the gift of pET21b vector used in E. coli construct 3 and Drs. Yefim Manevich, Michael Koval, Tom Sweitzer, Surafel Mulugeta, and Jhang Ho Pak for constructive discussions, Jamie Fisher for technical support, and Elaine Primerano for typing the manuscript.

REFERENCES
1. Kim, T.-S., Sunderesh, C. S., Feinstein, S. I., Dodia, C., Skach, W. R., Jain, M. K., Nagase, T., Seki, N., Ishikawa, K., Nomura, N., and Fisher, A. B. (1997) J. Biol. Chem. 272, 2542–2550.
2. Kim, T.-S., Dodia, C., Chen, X., Hennigan, B. B., Jain, M. K., Feinstein, S. I., and Fisher, A. B. (1998) Am. J. Physiol. 274, L750–L761.
3. Singh, A. K., and Shichi, H. (1996) J. Biol. Chem. 271, 26171–26178.
1-Cys Peroxiredoxin: A Bifunctional Enzyme

4. Fisher, A. B., Dodia, C., Manevich, Y., Chen, J.-W., and Feinstein, S. I. (1999) J. Biol. Chem. 274, 21326–21334
5. Kang, S.-W., Baines, I. C., and Rhee, S. G. (1998) J. Biol. Chem. 273, 6303–6311
6. Shichi, H., and Demar, J. C. (1990) Exp. Eye Res. 50, 513–520
7. Peshenko, I. V., Novoselov, V. I., Evdokimov, V. A., Nikolaev, Y. V., Shuvaeva, T. M., Lipkin, V. M., and Peseiko, E. E. (1996) FEBS Lett. 381, 12–14
8. Akiba, S., Dodia, C., Chen, X., and Fisher, A. B. (1998) Comp. Biochem. Physiol. Part B 120, 395–404
9. Jain, M. K., Yu, B.-Z., Gelb, M. H., and Berg, O. B. (1991) Biochemistry 30, 10256–10268
10. Fisher, A. B., Dodia, C., and Chander, A. (1994) Am. J. Physiol. 267, L335–L341
11. Jeffery, C. J. (1999) Trends Biochem. Sci. 24, 8–11
12. Tang, J., Kriz, R. W., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) J. Biol. Chem. 272, 8567–8575
13. Rice, S. Q. J., Southan, C., Boyd, H. P., Terrett, J. A., Macphee, C. H., Moores, K., Gloger, I. S., and Tew, D. G. (1998) Biochem. J. 330, 1309–1315
14. Wang, A., Deems, R. A., and Dennis, E. A. (1997) J. Biol. Chem. 272, 12725–12729
15. Derewenda, Z. S., and Sharp, A. M. (1993) Trends Biochem. Sci. 18, 20–25
16. Sato, J., Aoki, J., Nage, N., Tokio, K., Doi, T., Arai, H., and Inoue, K. (1997) J. Biol. Chem. 272, 2192–2198
17. Michiels, C., Raes, M., Toussaint, O., and Remacle, J. (1994) Free Rad. Biol. Med. 17, 235–248
18. Ursini, F., Maiorino, M., and Gregolin, C. (1985) Biochim. Biophys. Acta 839, 62–70
19. Forman, H. J., Rotman, E. I., and Fisher, A. B. (1983) Lab. Invest. 49, 148–153
20. Hurst, R., Bao, Y., Jemth, P., Mannervik, B., and Williamson, G. (1998) Biochem. J. 332, 97–100
21. Pathak, D., Ashley, G., and Ollis, D. (1991) Proteins 9, 267–279
22. Barford, D., Flint, A. J., and Tonks, N. K. (1994) Science 263, 1397–1403
23. Lewis, S. D., Johnson, F. A., and Shafer, J. A. (1976) Biochemistry 15, 5009–5017
24. Choi, S.-J., Kang, S. W., Yang, C.-H., Rhee, S. G., and Ryu, S.-E. (1998) Nature Struct. Biol. 5, 400–406
25. Fisher, A. B., and Dodia, C. (1996) J. Lipid Res. 37, 1057–1064
26. Chander, A., Johnson, R. G., Reichert, J., and Fisher, A. B. (1986) J. Biol. Chem. 261, 6126–6131
27. Fisher, A. B., Dodia, C., Chander, A., and Jain, M. K. (1992) Biochem. J. 288, 407–411
1-Cys Peroxiredoxin, a Bifunctional Enzyme with Glutathione Peroxidase and Phospholipase A₂ Activities
Jin-Wen Chen, Chandra Dodia, Sheldon I. Feinstein, Mahendra K. Jain and Aron B. Fisher

J. Biol. Chem. 2000, 275:28421-28427.
doi: 10.1074/jbc.M005073200 originally published online July 12, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M005073200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 27 references, 13 of which can be accessed free at http://www.jbc.org/content/275/37/28421.full.html#ref-list-1