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Molecular Cloning, Expression and Functional Characterization of a Peroxiredoxin from the Mole Cricket, *Gryllotalpa orientalis*

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Objectives

Peroxiredoxins are a family of antioxidant proteins ubiquitously found in all living organisms. A type of peroxidase enzyme, named thioredoxin peroxidase (TPx), that reduces H_2O_2 with the use of electrons from thioredoxin and contains two essential cysteines was identified in a wide variety of organisms ranging from prokaryotes to mammals. TPx homologs, termed peroxiredoxin (Prx), have also been identified and include several proteins, designated 1-Cys Prx, that contain only one conserved cysteine. The 1-Cys Prx has also been shown to reduce H_2O_2 with electrons provided by dithiothreitol.

Compared to mammalian peroxiredoxin genes, little is known about the insect Prx. Except for *Drosophila melanogaster*, genetic information on insect Prx is still unknown. Five peroxiredoxin genes have been identified in *D. melanogaster*: three of the genes fall into the 2-Cys subgroup, while the other two belong to the 1-Cys subgroup. These five proteins were shown to reduce H_2O_2 in the presence of dithiothreitol.

Until now insect Prx genes had been cloned from only *D. melanogaster*. Thus, our objective in initiating this study was to illustrate the cDNA cloning and molecular characterization of the Prx from the mole cricket, *Gryllotalpa orientalis*, which is a singly known species of the Family Gryllotalpidae in Korea, and distributed in Asia and many European and African countries. In this study, the molecular cloning, expression and functional characterization of the mole cricket, *G. orientalis* peroxiredoxin (GoPrx) are described.

Materials and Methods

Materials - The mole cricket, *Gryllotalpa orientalis*

Methods - cDNA library screening, nucleotide sequencing and data analysis, RNA isolation and Northern blot analysis, Construction of baculovirus transfer vector, Cell culture and construction of recombinant virus, SDS-polyacrylamide gel electrophoresis, Purification of recombinant Prx, Preparation of polyclonal antiserum and Western blot analysis, Determination of enzyme activity

Results and Discussion

A cDNA encoding the peroxiredoxin (Prx) from the mole cricket, *Gryllotalpa orientalis* was cloned and

characterized. The *G. orientalis* Prx (GoPrx) cDNA contains an open reading frame of 660 bp encoding 220 amino acid residues (Fig. 1 and 2). The GoPrx belongs to the 1-Cys subgroup of peroxiredoxin family (Fig. 2). The deduced amino acid sequence of the GoPrx cDNA showed 69% identity to *Drosophila melanogaster* DmPrx-2540 and 50% to *D. melanogaster* DmPrx-6005 (Fig. 3). Phylogenetic analysis confirmed a closer relationship of the deduced amino acid sequences of the GoPrx gene to the DmPrx-2540 and DmPrx-6005 of the 1-Cys PTx group (Fig. 3). The cDNA encoding GoPrx was expressed as a 27-kDa polypeptide in the baculovirus-infected insect Sf9 cells (Fig. 4) and the purified recombinant GoPrx was shown to reduce H_2O_2 in the presence of dithiothreitol (Fig. 5). Northern blot analysis revealed that GoPrx transcripts are present in all tissues examined, suggesting that GoPrx gene is expressed in most, if not all, body tissues (Fig. 6). The induction of GoPrx to H_2O_2 by Northern blot analysis showed that the level of GoPrx mRNA significantly increased during the H_2O_2 exposure (Fig. 7).

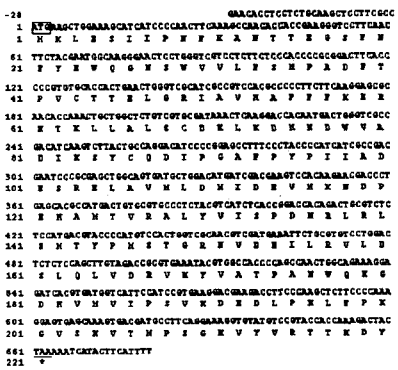

Fig. 1. The nucleotide and deduced protein sequence of the GoPrx cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. In the cDNA sequence, the polyadenylation sequence is underlined.

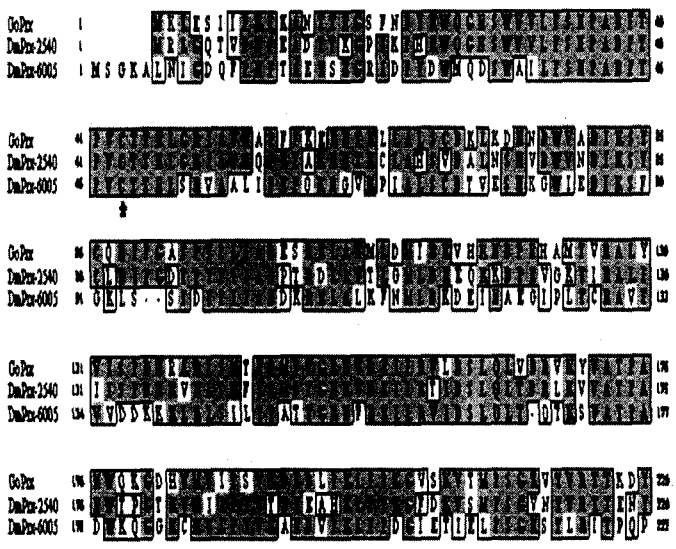

Fig. 2. Comparison of the deduced amino acid sequences of GoPrx with the *D. melanogaster* Prx. Residues are numbered according to the aligned *D. melanogaster* Prx sequences, and invariant residues are shaded black. The conserved cysteine residue is shown by asterisk. The *D. melanogaster* Prx sequences were taken from the following sources: DmPrx-2540 (AAF58797) and DmPrx-6005 (NM_523463).

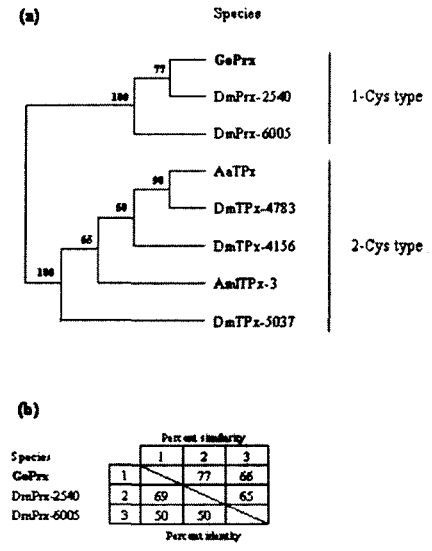

Fig. 3. Phylogenetic relationship among insect peroxiredoxin sequences. (a) A maximum parsimony analysis for the GoPrx and the other known insect Prx and TPx sequences. The accession numbers of the sequences in the GenBank are as follows: DmPrx-2540 (AAF58797), DmPrx-6005 (NM_523463), DmTPx-1 (Q9V3P0), AaTPx (AAL37254), DmTPx-2 (AAF42986), AmlTPx-3 (AAP93584), and DmTPx-3 (AAG41976). The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates. (b) Pairwise identities and similarities of the deduced amino acid sequence of GoPrx among insect Prx sequences.

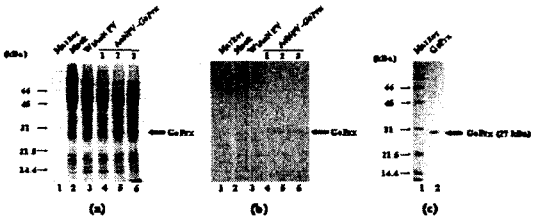

Fig. 4. Expression of the GoPrx in recombinant baculovirus-infected insect Sf9 cells and purification of its recombinant GoPrx. Sf9 cells were mock-infected (lane 2) or infected with the wild-type AcNPV (lane 3) and the recombinant AcNPV (lane 4, 5, and 6) at an MOI of 5 PFU per cell. Cells were collected at 1 (lane 4), 2 (lanes 3 and 5) and 3 (lane 6) days p.i. Total cellular lysates were subjected to 12% SDS-PAGE (a), electroblotted and incubated with antiserum to recombinant GoPrx (b). The arrow on the right of the panel indicates the 27 kDa recombinant GoPrx polypeptide. Molecular weight standards were used as size marker (lane 1). (c) Purification of the recombinant GoPrx expressed in baculovirus-infected insect cells. The recombinant GoPrx purified by using FPLC techniques was analyzed by 12% SDS-PAGE. The arrow on the right of the panel indicates the purified recombinant GoPrx polypeptide of 27 kDa.

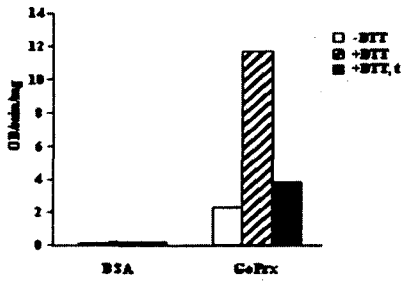

Fig. 5. Hydrogen peroxide elimination by the GoPrx expressed in recombinant baculovirus-infected insect Sf9 cells. H_2O_2 degradation was monitored at 240 nm and the differences in OD readingswere plotted on the Y axis. The reactions were carried out with 10 μg of each purified GoPrx or 1.25 mg BSA in the absence (-DTT) or presence (+DTT) of 10 mM DTT or in the presence of DTT and the same amount of thermo-inactivated GoPrx [boiled for 5 min in 0.5% SDS (+DTT, t)]. Heat-inactivated GoPrx and BSA were used as negative controls.

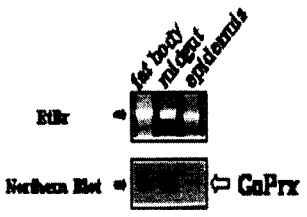

Fig. 6. Northern blot analysis of GoPrx. Total RNA was isolated from the fat body (lane 1), midgut (lane 2), and epidermis (lane 3), respectively (upper panel). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis, transferred on to a nylon membrane, and hybridized with radiolabelled 660 bp GoPrx cDNA (lower panel). Transcripts are indicated on the right side of the panel by arrow.

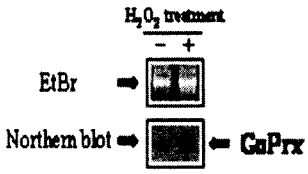

Fig. 7. Induction of GoPrx by exposure of H_2O_2. The *G. orientalis* was injected without (lane 1) or with (lane 2) 20 μM H_2O_2 per each larva. After 1 h-treatment, total RNA was isolated from the fat body, respectively. The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred on to a nylon membrane, and hybridized with radiolabelled 660 bp GoPrx cDNA (lower panel). Transcripts are indicated on the right side of the panel by arrow.

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