Int. J. Indust. Entomol. Vol. 4, No. 2, 2002, pp. 101~107

International Journal of *Industrial Entomology*

cDNA Sequence and mRNA Expression of a Novel Peroxiredoxin from the Firefly, *Pyrocoelia rufa*

Kwang Sik Lee, Seong Ryul Kim, Hung Dae Sohn and Byung Rae Jin*

College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.

(Received 11 March 2002; Accepted 5 April 2002)

We describe here the cDNA sequence and mRNA expression of a novel family of the antioxidant protein, peroxiredoxin, from the firefly, *Pyrocoelia rufa*. The 555 bp cDNA sequence codes for a 185 amino acid protein with a calculated molecular mass of approximately 21 kDa. The deduced protein of *P. rufa* peroxiredoxin gene contains two conserved cysteine residues. Alignment of the deduced protein of *P. rufa* peroxiredoxin gene showed 71.1% protein sequence identity to known insect *Drosophila melanogaster* peroxiredoxin. Northern blot analysis revealed that the *P. rufa* peroxiredoxin is specifically expressed in the fat body of *P. rufa* larvae.

Key words : Insect, Firefly, Antioxidant protein, Peroxiredoxin, *Pyrocoelia rufa*, cDNA sequence, Phylogeny

Introduction

Peroxiredoxin (Prx) protein is the antioxidant protein that reduces H_2O_2 with the use of electrons provided by thioredoxin (Chae *et al.*, 1994a). Initially identified in yeast, Prx has been discovered in prokaryotes and eukaryotes (Chae *et al.*, 1994b). More than 40 proteins from a wide variety of species show sequence similarity to Prx. Peroxiredoxins are considered to be involved in oxidative stress protection mechanisms but also in cell differentiation, proliferation, immune response, and apoptosis, as well as activation of viruses, notably HIV, from latency (Holmgren, 1989; Ichimiya *et al.*, 1997; Kang *et al.*, 1998a; Prosperi *et al.*, 1993; Rabilloud *et al.*, 1995; Zhang *et al.*, 1997).

*To whom correspondence should be addressed.
College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.
Tel: +82-51-200-7594; Fax: +82-51-200-7553;
E-mail: brjin@mail.donga.ac.kr

In insect, Prx have firstly been identified in *Drosophila melanogaster* (Rodriguez *et al.*, 2000). Two peroxiredoxins (Jafrac 1 and Jafrac 2) of *Drosophila* contain two conserved cysteine residues and share homology with other Prxs that extends over the entire sequence and ranges between 47% and 76%.

In this study, we report the cDNA sequence and mRNA expression of a novel family of the antioxidant protein, Prx, from the firefly, *Pyrocoelia rufa*. The characterization for novel family of Prx in Coleoptera is discussed.

Materials and Methods

Insects

The larvae of the firefly, *Pyrocoelia rufa*, were collected at Miryang, Kyungnam province in Korea. The live larvae were directly used in this study.

cDNA library screening , nucleotide sequencing and data analysis

A cDNA library (Lee *et al.*, 2000) was constructed from the whole body of *P. rufa* larvae. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program.

MacVector (ver. 6.5) was used to align the amino acid sequences of Prx gene. Including the eighteen GenBank-registered amino acid sequences of Prx genes, phylogenetic analysis among Prx genes was performed using

PAUP (Phylogenetic Analysis using Parsimony) version 3.0 (Swofford, 1990). The tree was obtained by bootstrap analysis with the option of heuristic search (1,000 replications). Outgroup was chosen as *Xerophyta viscosa* Prx gene on the basis of the sequence homology obtained by pairwise comparison. The accession numbers of the sequences in the GenBank are as follows: firefly *P. rufa* (this study), fruit fly *D. melanogaster* (AY051983), *Sinorhizobium meliloti* (AL591785), *Agrobacterium tumefaciens* (NC003062), *Brucella melitensis* (NC003317), *Vibrio cholerae* (AE004215), *Ralstonia solanacearum* (AL646072), rice *Oryza sativa* (AF203879), *Capsicum annuum* (AF 442385), Chinese cabbage *Brassica rapa pekinensis* (AF 133302), *Arabidopsis thaliana* (NM105269), pig *Sus scrofa* (AF110735), Norway rat *Rattus norvegicus* (NM053610), house mouse *Mus musculus* (NM012021), baboon *Papio hamadryas* (AF110734), African green monkey *Cercopithecus aethiops* (AF110736), human *Homo sapiens* (AF 110731), cow *Bos taurus* (AF305564), and *X. viscosa* (AF484696).

RNA isolation and Northern blot analysis

Ten larvae of *P. rufa* were dissected under the Stereomicroscope (Zeiss, Jena, germany). Individual samples such as midgut and fat body were harvested and washed twice with PBS (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNAs were isolated from the whole body, mid gut, and fat body of the *P. rufa* larvae by using the Total RNA Extraction Kit (Promega, Madison, WI). Total RNAs (10 μg/lane) from the *P. rufa* were denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2 × PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the Prx gene transcripts was 555 bp Prx gene cloned in this study and labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C, and finally exposed to X-ray film. For rehybridization, the membrane was washed for 20 min at room temperature in sterile millipore water. Then the membrane was washed overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS in order to remove the hybridized probe. The membrane was then rehybridized to [α-^{32}P] dCTP-labeled 16S rRNA probe (Lee *et al.*, 2000). The 16S rRNA gene was used as an internal loading control.

```
1   ATGCGCTTGCTAGGAGTCATCAAAACGCTGGGGAGAATTGTATCAATTGTTCCTCAACGC
1    M  R  L  L  G  V  I  K  T  L  G  R  I  V  S  I  V  P  Q  R

61  CTTATCCTTAGGTCGTATTTAATCACCGCGGCTATGCCAATTAAGGAAGGTGAATCTTTA
21   L  I  L  R  S  Y  L  I  T  A  A  M  P  I  K  E  G  E  S  L

121 CCAAACATTGATCTCTTCGAGGAGACTCCGGCAAATAAAGTTAACCTAAATGAACTATCC
41   P  N  I  D  L  F  E  E  T  P  A  N  K  V  N  L  N  E  L  S

181 GCAGGAAAAAAGATCATCGTCTTCGCAGTTCCCGGTGCCTTCACTCCGGGTTGTTCAAAG
61   A  G  K  K  I  I  V  F  A  V  P  G  A  F  T  P  G  C  S  K
                                                      △

241 ACTCATCTACCCGGCTATGTAAAAAAGGCGGAAGAATTAAAATTGCAAGGTATCAGCGAA
81   T  H  L  P  G  Y  V  K  K  A  E  E  L  K  L  Q  G  I  S  E

301 ATTGCATGTGTGGCTGTTAACGATCCTTTCGTAATGTCGGCATGGGGCAAGGAACACAAT
101  I  A  C  V  A  V  N  D  P  F  V  M  S  A  W  G  K  E  H  N
        △

361 GTAAACGGTAAGATACGCATGCTGGCCGATACGCAGGCGACATTTACCAAGTCCATTGAT
121  V  N  G  K  I  R  M  L  A  D  T  Q  A  T  F  T  K  S  I  D

421 CTCGGGCAAGATCTGGCTCCGCTTGGTGGTTATCGTAGCAAACGGTATTCGATGGTTGTC
141  L  G  Q  D  L  A  P  L  G  G  Y  R  S  K  R  Y  S  M  V  V

481 GAAAATGGTAAAGTTCAATCTTTACAAGTCGACCCGATGGTACCGGATATCATGCTCGTG
161  E  N  G  K  V  Q  S  L  Q  V  D  P  M  V  P  D  I  M  L  V

541 GTGGAGCTATCAGTATAA
181  V  E  L  S  V  *
```

Fig. 1. The nucleotide and deduced amino acid sequences of *P. rufa* Prx gene. The start codon of ATG is boxed and the termination codon is asterisked. Two cysteine residues are marked by open triangles.

Results and Discussion

Construction of a cDNA library was prepared from the whole body of *P. rufa* larvae (Lee *et al.*, 2000). The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the *P. rufa* ESTs. Of these ESTs, one exhibited similarity to the reported Prx genes. The nucleotide and deduced amino acid sequences of a full length cDNA encoding the *P. rufa* Prx is presented in Fig. 1. The complete Prx cDNA sequence comprised of 555 bp with 185 amino acid residues with a calculated molecular mass of approximately 21 kDa.

A multiple sequence alignment of the deduced protein sequence of *P. rufa* Prx gene with other Prx sequences is shown in Fig. 2. Alignment of the *P. rufa* Prx sequences with those for Prx from several other species indicates the extent of the identity that exists. The Prx active site (Verdoucq *et al.*, 1999; Zhou *et al.*, 2000) is conserved in the surrounding region of the first cystein residue. The Prx proteins fall largely into the two subfamilies of one cysteine Prx and two cysteins Prx proteins (Kang *et al.*, 1998b). The Prx proteins use the sulfhydryl group of cysteine as a strong nucleophile in their attack on hydrogen peroxide and hydroxyl radicals (Choi *et al.*, 1998). The deduced protein of *P. rufa* Prx gene has two conserved cysteine residues over the entire sequence, indicating that *P. rufa* Prx is two cysteins Prx protein type. The *Drosophila* Prx also contains two conserved cysteine residues (Rodriguez *et al.*, 2000).

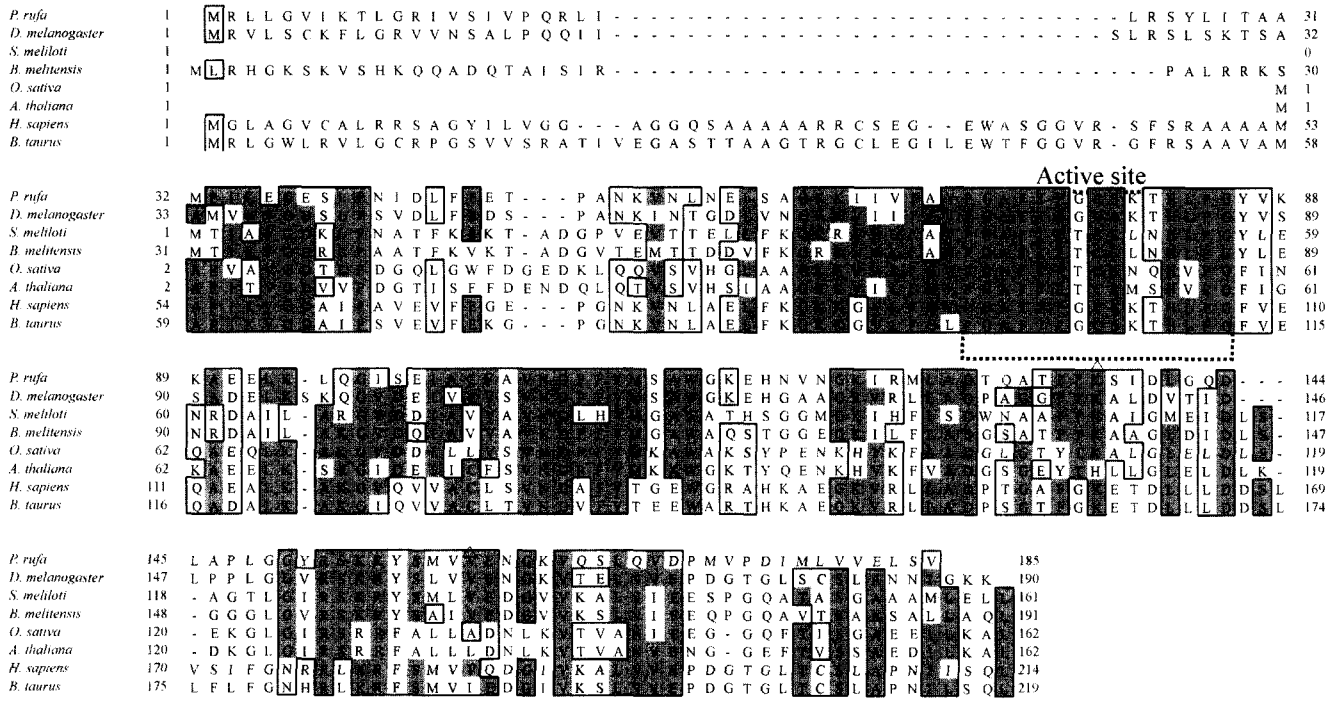

Fig. 2. Alignment of deduced amino acid sequences of *P. rufa* Prx gene with other known Prxs. In solid box are the residues that are identical to those in *P. rufa* Prx. The Prx active site in the Prx sequence is lined. Two conserved cysteine residues are marked by open triangles.

Table 1. Pairwise comparisons among amino acid sequences of the *P. rufa* peroxiredoxin gene and the known peroxiredoxin gene

Species	GenBank No.	1	2	3	4	5	6	7	8	9	10
1. P. rufa		-	0.289	0.423	0.450	0.440	0.443	0.471	0.471	0.467	0.464
2. D. melanogaster	AY051983	84	-	0.419	0.450	0.433	0.433	0.454	0.426	0.426	0.426
3. V. cholerae	AAF94508	123	122	-	0.309	0.278	0.399	0.285	0.292	0.309	0.299
4. S. meliloti	CAC45487	131	131	90	-	0.110	0.247	0.323	0.309	0.313	0.351
5. A. tumefaciens	NP_353803	128	126	81	32	-	0.220	0.299	0.313	0.306	0.337
6. B. melitensis	NP_540373	129	126	116	72	64	-	0.412	0.409	0.409	0.440
7. R. solanacearum	CAD16545	137	132	83	94	87	120	-	0.320	0.313	0.320
8. O. sativa	AF203879	137	124	85	90	91	119	93	-	0.141	0.137
9. C. annuum	AF442385	136	124	90	91	89	119	91	41	-	0.103
10. B. rapa perkinensis	AF133302	135	124	87	102	98	128	93	40	30	-
11. A. thaliana	AF332463	136	128	91	105	102	131	94	43	37	12
12. S. scrofa	AF110735	115	106	99	102	96	129	114	99	100	100
13. R. norvegicus	AF110732	139	120	148	153	151	151	165	149	150	150
14. M. musclus	AF197951	134	115	146	149	147	150	161	144	145	145
15. P. hamadryas	AF110734	131	122	154	152	149	149	163	151	150	152
16. C. aethiops	AF110736	132	121	153	152	149	149	163	151	150	152
17. H. sapiens	AF110731	132	119	152	151	148	149	163	150	149	151
18. B. taurus	AF305564	135	124	159	157	153	149	169	156	154	157
19. X. viscosa	AF484696	225	213	188	195	192	221	203	193	191	191

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

Table 1. (*Continued*)

Species	GenBank No.	11	12	13	14	15	16	17	18	19
1. P. rufa		0.467	0.395	0.478	0.460	0.450	0.454	0.454	0.464	0.773
2. D. melanogaster	AY051983	0.440	0.364	0.412	0.395	0.419	0.416	0.409	0.426	0.732
3. V. cholerae	AAF94508	0.313	0.340	0.509	0.502	0.529	0.526	0.522	0.546	0.646
4. S. meliloti	CAC45487	0.361	0.351	0.526	0.512	0.522	0.522	0.519	0.540	0.670
5. A. tumefaciens	NP_353803	0.351	0.33	0.519	0.505	0.512	0.512	0.509	0.526	0.660
6. B. melitensis	NP_540373	0.450	0.443	0.519	0.515	0.512	0.512	0.512	0.512	0.759
7. R. solanacearum	CAD16545	0.323	0.392	0.567	0.553	0.560	0.560	0.560	0.581	0.698
8. O. sativa	AF203879	0.148	0.34	0.512	0.495	0.519	0.519	0.515	0.536	0.663
9. C. annuum	AF442385	0.127	0.344	0.515	0.498	0.515	0.515	0.512	0.529	0.656
10. B. rapa perkinensis	AF133302	0.041	0.344	0.515	0.498	0.522	0.522	0.519	0.540	0.656
11. A. thaliana	AF332463	-	0.354	0.526	0.509	0.536	0.536	0.533	0.550	0.660
12. S. scrofa	AF110735	103	-	0.237	0.213	0.241	0.241	0.230	0.265	0.649
13. R. norvegicus	AF110732	153	69	-	0.076	0.172	0.168	0.168	0.220	0.818
14. M. musclus	AF197951	148	62	22	-	0.175	0.172	0.172	0.203	0.811
15. P. hamadryas	AF110734	156	70	50	51	-	0.007	0.045	0.210	0.825
16. C. aethiops	AF110736	156	70	49	50	2	-	0.045	0.206	0.821
17. H. sapiens	AF110731	155	67	49	50	13	13	-	0.213	0.821
18. B. taurus	AF305564	160	77	64	59	61	60	62	-	0.845
19. X. viscosa	AF484696	192	189	238	236	240	239	239	246	-

The *P. rufa* Prx showed 71.1% protein sequence identity to the *D. melanogaster* Prx (Table 1). Furthermore, *P. rufa* Prx shows sequence similarity to other known Prxs from a wide variety of species, ranging from prokaryotes to mammals. This result was similar to *Drosophila* Prx that shares homology with other Prxs that extends over the entire sequence and ranges between 47% and 76% (Rodriguez *et al.*, 2000).

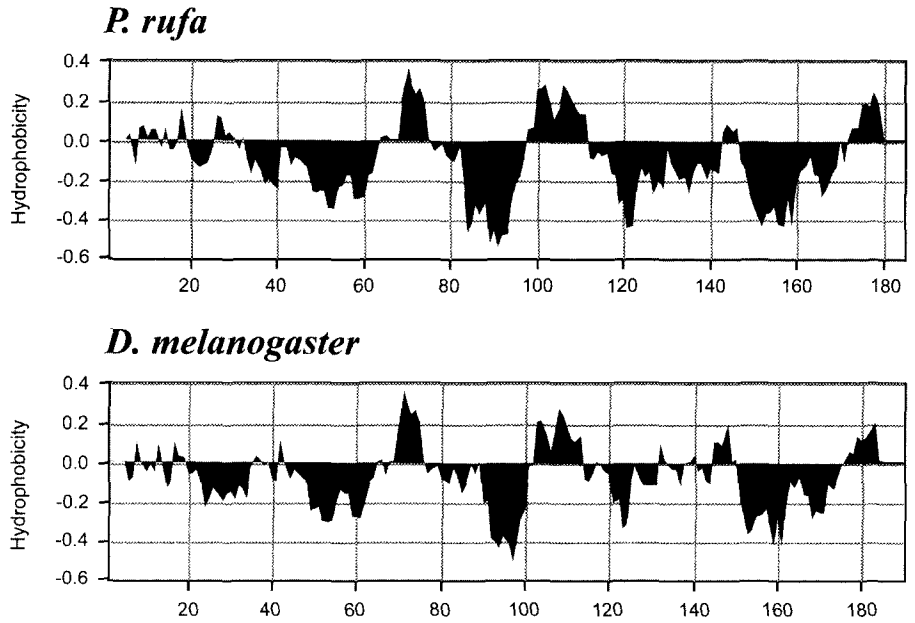

Fig. 3. The hydropathy profile of *P. rufa* Prx and known insect *Drosophila* Prx. Hydropathic analysis was done as described by Kyte and Doolittle (1982).

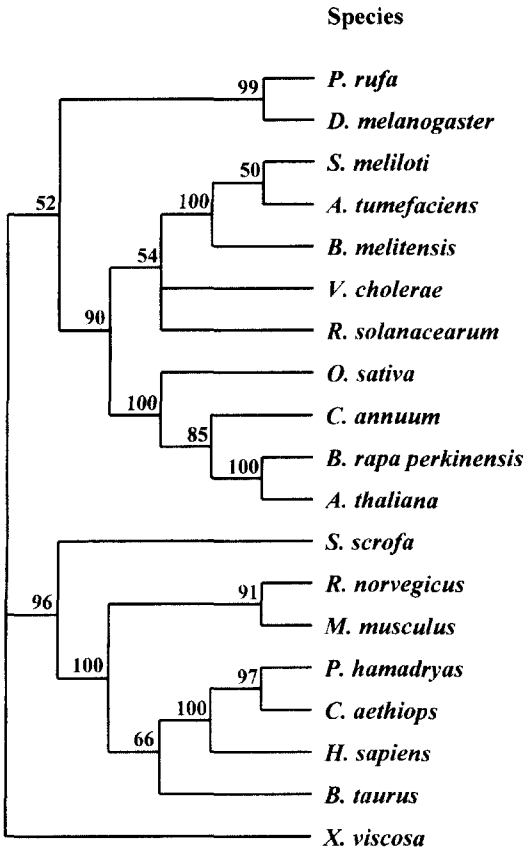

Fig. 4. A phylogenetic tree for aligned amino acid sequences of the *P. rufa* Prx and the known Prxs. The sequences were extracted from; *P. rufa* (this study), *D. melanogaster* (Stapleton *et al.*, 2001), *S. meliloti* (Capela *et al.*, 2001), *A. tumefaciens* (Goodner *et al.*, 2001), *B. melitensis* (DelVecchio *et al.*, 2002), *V. cholerae* (Heidelberg *et al.*, 2000), *R. solanacearum* (Salanoubat *et al.*, 2001), *O. sativa* (Alegre *et al.*, 2000), *C. annuum* (Hwang and Do, 2002), *B. rapa pekinensis* (Choi *et al.*, 1999), *A. thaliana* (Town *et al.*, 2002), *S. scrofa* (Knoops *et al.*, 1999), *R. norvegicus* (Knoops *et al.*, 1999), *M. musculus* (Yamashita *et al.*, 1999), *P. hamadryas* (Knoops *et al.*, 1999), *C. aethiops* (Knoops *et al.*, 1999), *H. sapiens* (Knoops *et al.*, 1999), *B. taurus* (Leyens *et al.*, 2001), and *X. viscosa* (Mowla *et al.*, 2002). 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. Outgroup was chosen as *X. viscosa* on the basis of the sequence homology by pairwise comparison.

The hydropathy plot of *P. rufa* Prx was analyzed by the Kyte and Doolittle method (Kyte and Doolittle, 1982), and compared with known insect *Drosophila* Prx showing a high homology. As shown in Fig. 3, hydropathy analysis suggests the profile of *P. rufa* Prx is similar to insect *Drosophila* Prx, indicating that *P. rufa* Prx is hydrophilic. A phylogenetic tree was constructed using the protein

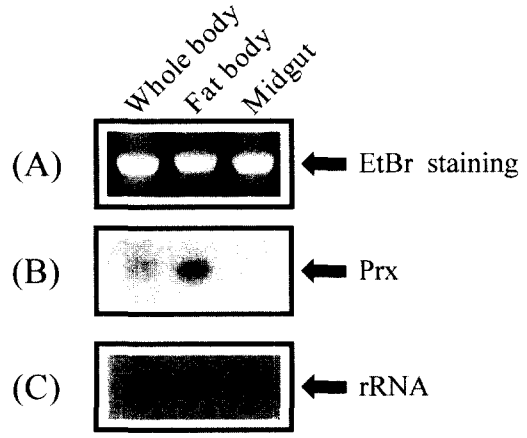

Fig. 5. Northern blot analysis of the *P. rufa* Prx gene. Total RNAs were isolated from the whole body (lane 1), fat body (lane 2) and mid gut (lane 3), respectively. The RNAs were separated by 1.0% formaldehyde agarose gel electrophoresis (A), transferred on to a nylon membrane, and hybridized with the radiolabelled *P. rufa* Prx gene (B). The 16S rRNA gene was used as an internal loading control (C). Transcripts of the *P. rufa* Prx gene are indicated on the right of panel by arrow.

sequences of known Prx genes (Fig. 4). The phylogenetic analysis revealed that the deduced protein sequence of *P. rufa* Prx gene formed a subgroup with an insect *Drosophila* Prx.

To confirm the expression of the *P. rufa* Prx gene at the transcriptional level, the Northern blot analysis was carried out using the mRNA prepared from the mid gut and fat body, respectively (Fig. 5). A hybridization signal was detected as a single band in mRNA from the whole body as a positive control and fat body. The Northern hybridization revealed that *P. rufa* Prx is specifically expressed in the fat body of *P. rufa* larvae.

In conclusion, we report the cDNA sequence and mRNA expression of Prx in the firefly, *P. rufa*. In this study, molecular characterization of *P. rufa* Prx will expand our understanding on the insect Prxs.

Acknowledgements

This paper was supported by the Dong-A University Research Fund in 2001.

References

Alegre, O., M. Antolin and S. Imperial (2000) Characterization of a new type of peroxiredoxin from *Oryza sativa*. *Genbank accession number AF203879*.

Capela, D., F. Barloy-Hubler, J. Gouzy, G. Bothe, F. Ampe, J. Batut, P. Boistard, A. Becker, M. Boutry, E. Cadieu, S. Dreano, S. Gloux, T. Godrie, A. Goffeau, D. Kahn, E. Kiss, V. Lelaure, D. Masuy, T. Pohl, D. Portetelle, A. Puehler, B. Purnelle, U. Ramsperger, C. Renard, P. Thebault, M. Vandenbol, S. Weidner and F. Galibert (2001) Analysis of the chromosome sequence of the legume symbiont *Sinorhizobium meliloti* strain 1021. *Proc. Natl. Acad. Sci. USA* **98**, 9877-9882.

Chae, H. Z., J. S. Chung and S. G. Rhee (1994a) Thioredoxin-dependent peroxide reductase from yeast. *J. Biol. Chem.* **269**, 27670-27678.

Chae, H. Z., K. Robison, L. B. Poole, G. Church, G. Storz and S. G. Rhee (1994b) Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. *Proc. Natl. Acad. Sci. USA* **91**, 7017-7021.

Choi, H. J., S. W. Kang, C. H. Yang, S. G. Rhee and S. E. Ryu (1998) Crystal structure of a novel human peroxidase enzyme at 2.0 angstrom resolution. *Nat. Struct. Biol.* **5**, 400-406.

Choi, Y. O., N. E. Cheong, K. O. Lee, B. G. Jung, C. H. Hong, J. H. Jeong, Y. H. Chi, K. Kim, M. J. Cho and S. Y. Lee (1999) Cloning and expression of a new isotype of the peroxiredoxin gene of Chinese cabbage and its comparison to 2Cys-peroxiredoxin isolated from the same plant. *Biochem. Biophys. Res. Commun.* **258**, 768-771.

DelVecchio, V. G., V. Kapatral, R. J. redkar, G. Patra, C. mujer, T. Los, N. Ivanova, I. Andersob, A. Bhattacharyya, A. Lykidis, G. Reznik, L. Jablonski, N. Larsen, M. DSouza, A. Bernal, M. Mazur, E. Goltsman, E. Selkov, P. H. Elzer, S. Hagius, D. OCallaghan, J. J. Letesson, R. Haselkorn, N. Kyrpides and R. Overbeek (2002) The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proc. Natl. Acad. Sci. USA* **99**, 443-448.

Goodner, B., G. Hinkle, S. Gattung, N. Miller, M. Blanchard, B. Qurollo, K. Houmiel, J. Gordon, M. Vaudin, O. Iartchouk, A. Epp, F. Liu, C. Wollam, M. Allinger, D. Doughty, C. Scott, C. Lappas, B. Markelz, C. Flanagan, C. Crowell, J. Gurson, C. Lomo, C. Sear, G. Strub, C. Cielo and S. Slater (2001) Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science* **294**, 2323-2328.

Heidelberg, J. F., J. A. Eisen, W. C. Nelson, R. A. Clayton, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, L. Umayam, S. R. Gill, K. E. Nelson, T. D. Read, H. Tettelin, D. Richardson, M. D. Ermolaeva, J. Vamathevan, S. Bass, H. Qin, I. Dragoi, P. Sellers, L. McDonald, T. Utterback, R. D. Fleishmann, W. C. Nierman and O. White (2000) DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* **406**, 477-483.

Hwang, B. K. and H. M. Do (2002) Pepper ascorbate peroxidase and thioredoxin peroxidase genes regulate H202 accumulation and peroxidase activity during the bacteria-induced hypersensitive reaction in *Capsicum annuum*. *Genbank accession number AF442385*.

Ichimiya, S., J. G. Davis, D. M. ORourke, M. Katsumata and M. I. Greene (1997) Murine thioredoxin peroxidase delays neuronal apoptosis and is expressed in areas of the brain most susceptible to hypoxic and ischemic injury. *DNA Cell Biol.* **16**, 311-321.

Kang, S. W., H. Z. Chae, M. S. Seo, K. Kim, I. C. Baines and S. G. Rhee (1998a) Mammalian peroxiredoxin isoforms can reduce hydrogen peroxide generated in response to growth factors and tumor necrosis factors-α. *J. Biol. Chem.* **273**, 6297-6302.

Kang, S. W., I. C. Baines and S. G. Rhee (1998b) Characterization of a mammalian peroxiredoxin that contains one conserved cysteine. *J. Biol. Chem.* **273**, 6303-6311.

Knoops, B., A. Clippe, C. Bogard, K. Arsalane, R. Wattiez, C. Hermans, E. Duconseille, P. Falmagne and A. Bernard (1999) Cloning and characterization of AOEB166, a novel mammalian antioxidant enzyme of the peroxiredoxin family. *J. Biol. Chem.* **274**, 30451-30458.

Kyte, J. and R. F. Doolittle (1982) A simple method for displaying hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132.

Lee, K. S., J. S. Bae, T. W. Goo, S. E. Kim, J. G. Kim, H. D. Sohn and B. R. Jin (2000) Analysis of expressed sequence tags of the firefly, *Pyrocoelia rufa*. *Int. J. Indust. Entomol.* **1**, 165-169.

Leyens, G., I. Donnay and B. Knoops (2001) Cloning of 4 new bovine peroxiredoxins, and screening of the complete peroxiredoxin family in different bovine tissues. *GenBank accession number AF305564*.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacryamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Mowla, S. B., J. A. Thomson, J. M. Farrant and S. G. Mundree (2002) A novel stress-inducible antioxidant enzyme identified from the resurrection plant *Xerophyta viscosa* Baker. *GenBank accession number AF484696*.

Prosperi, M. T., D. Ferbus, I. Karczinski and G. Goubin (1993) A human cDNA corresponding to a gene overexpressed during cell proliferation encodes a product sharing homology with amoebic and bacterial proteins. *J. Biol. Chem.* **268**, 11050-11056.

Rabilloud, T., R. Berthier, M. Vincon, D. Ferbus, G. Goubin and J. J. Lawrence (1995) Early events in erythroid differentiation: accumulation of the acidic peroxidoxin (PRP/TSA/NKEF-B). *Biochem. J.* **312**, 699-705.

Rodriguez, J., M. Agudo, J. V. Damme, J. Vandekerckhove and J. F. Santaren (2000) Polypeptides differentially expressed in imaginal disc define the peroxiredoxin family of genes in *Drosophila*. *Eur. J. Biochem.* **267**, 487-497.

Salanoubat, M., S. Genin, F. Artiguenave, J. Gouzy, S. Mangenot, M. Arlat, A. Billault, P. Brottier, J. C. Camus, L. Cattolico, M. Chandler, N. Choisne, C. Claudel-Renard, S.

Cunnac, N. Demange, C. Gaspin, M. Lavie, A. Moisan, C. Robert, W. Saurin, T. Schiex, P. Siguier, P. Thebault, M. whalen, P. Wincker, M. Levy, J. Weissenbach and C. A. Boucher (2001) Genome sequence of the plant pathogen *Ralstonia solanacearum. Genbank accession number AL 646072.*

Swofford, D. L. (1990) PAUP: phylogenetic analysis using parsimony, ver. 3.0. Illinois Natural History Survey, Champaign (on disk).

Town, C. D., B. J. Haas, D. Wu, R. Maiti, L. I. Hannick, A. P. Chan, L. J. Tallon, T. Rooney, T. R. Utterback, S. E. VanAken, T. V. Feldblyum, O. White and C. M. Fraser (2002) Arabidopsis thaliana chromosome 1 CHR1v1215 2001 genomic sequence. GenBank accession number NM 105269.

Verdoucq, L., F. Vignols, J. P. Jacquot, Y. Chartier and Y. Meyer (1999) In vivo characterization of a thioredoxin target protein defines a new peroxiredoxin family. J. Biol. Chem. **274**, 19714-19722.

Yamashita, H., S. Avraham, S. Jiang, R. London, P. P. Van Veldhoven, S. Subramani, R. A. Rogers and H. Avraham (1999) Characterization of human and murine PMP20 peroxisomal proteins that exhibit antioxidant activity in vitro. J. Biol. Chem. **274**, 29897-29904.

Zhang, P., B. Liu, S. W. Kang, M. S. Seo, S. G. Rhee and L. M. Obeid (1997) Thioredoxin peroxidase is a novel inhibitor of apoptosis with a mechanism distinct from that of Bcl-2. J. Biol. Chem. **272**, 30615-30618.

Zhou, Y., K. H. Kok, A. C. S. Chun, C. M. Wong, H. W. Wu, M. C. M. Lin, P. C. W. Fung, H. F. Kung and D. Y. Jin (2000) Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. Biochem. Biophys. Res. Commun. **268**, 921-927.