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Cloning and Characterization of the Cu,Zn Superoxide Dismutase (SOD1) cDNA from the Spider, *Araneus ventricosus*

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A Cu,Zn superoxide dismutase (SOD1) cDNA was cloned from the spider, *Araneus ventricosus*. The *A. ventricosus* SOD1 (AvSOD1) cDNA contains an open reading frame of 495 bp encoding 165 amino acid polypeptide with a predicted molecular mass of 17,114 Da and pI of 6.55, and possesses the typical metal binding ligands of six histidines and one aspartic acid common to SOD1s. The deduced amino acid sequence of the AvSOD1 cDNA showed 51% identity to *Ceratitis capitata* SOD1, and 50% to SOD1 sequences of both *Drosophila melanogaster* and *Chymomyza amoena*. Northern blot analysis revealed the presence of AvSOD1 transcripts in all tissues examined.

Key words: Antioxidant protein gene, *Araneus ventricosus*, Cu,Zn superoxide dismutase (SOD1), Spider

Introduction

The first line of defense against oxidative stress resulting from the generation of reactive oxygen species (ROS), which is a significant source of cellular and DNA damage (Mount, 1996; Dalton *et al.*, 1999), includes the enzymatic activity of superoxide dismutase (SOD) that catalyzes the disproportionation of superoxide to hydrogen peroxide and water (McCord and Fridovich, 1969; Fridovich, 1986). SOD mainly removes highly toxic O_2^- and also prevents O_2^- mediated reduction of iron and subsequent OH^- generation. One of the SOD enzymes, Cu,Zn SOD (SOD1) is found primarily in the cytosol of eukaryotes (Crapo *et al.*, 1992). Mn-SOD (SOD2) is present in the mitochondria of both prokaryotes and eukaryotes, and Fe-SOD is found in both eubacteria and archaebacteria (Fridovich, 1995).

The SOD1 enzyme, which binds on one copper and one zinc ion and displays the Greek Key β-barrel fold (Tainer, 1982), was found in the green algae and in all higher eukaryote species (Fridovich, 1995). Molecular characterization of SOD1 has been investigated in various species. Mutants of the yeast *Saccharomyces cerevisiae* and the filamentous fungus *Neurospora crassa* lacking SOD1 are sensitive to oxygen and superoxide generating agents (Chary *et al.*, 1994; Jamieson *et al.*, 1994). Furthermore, ROS is considered to be a main proximate cause of aging and, accordingly, one of the antioxidant enzymes thought to be involved in lifespan extension is SOD1. SOD1 overexpression was found to extend lifespan in *S. cerevisiae* (Fabrizio *et al.*, 2003). In the fruit fly *Drosophila melanogaster*, increased lifespan has been obtained by overexpression of SOD1 (Orr and Sohal, 1994; Sohal *et al.*, 1995; Parkes *et al.*, 1998; Sun and Tower, 1999). Most recently, however, it has been shown that long-lived queens of the ant *Lasius niger* have a lower level of SOD1 expression than workers and males, indicating that increased expression of SOD1 is not required for the evolution of extreme lifespan (Parker *et al.*, 2004).

In arthropod, SOD1 genes have been isolated only from several insect species of four orders such as Diptera, Lepidoptera, Hymenoptera, and Orthoptera. In order to obtain molecular information of the spider, *Araneus ventricosus*, we report the cDNA cloning and characterization of the SOD1 gene from *A. ventricosus* (AvSOD1) and compared the amino acid sequences with other SOD1s. Also, we found that the AvSOD1 was ubiquitously expressed in *A. ventricosus* adult. This is the first report on the cDNA cloning and characterization of an SOD1 gene from the spider.

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Materials and Methods

Animals

The spider, *Araneus ventricosus*, was collected at Namhae, Kyungnam province in Korea. The live spider was directly used in this study.

cDNA library screening, nucleotide sequencing and data analysis

A cDNA library (Chung *et al.*, 2001) was constructed using whole bodies of the spider *A. ventricosus*. 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, Madison, WI). 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 (www.ncbi.nlm.nih.gov). GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program (www.ncbi.nlm.nih.gov/BLAST). MacVector (ver. 6.5) was used to align the amino acid sequences of SOD1 gene. With the three GenBank-registered SOD1 amino acid sequences, phylogenetic analysis was performed using PAUP* (Phylogenetic Analysis using Parsimony) version 4.0 (Swofford, 2000). The accession numbers of the sequences in the GenBank are as follows: *Araneus ventricosus* (AY786991; this study), *Ceratitis capitata* (M76975), *Drosophila melanogaster* (AY071435), and *Chymomyza amoena* (X61687).

RNA isolation and Northern blot analysis

The *A. ventricosus* adult was dissected under the Stereomicroscope (Zeiss, Jena, Germany), individual samples such as head part, gut, silk gland, fat body, and epidermis 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 RNA was isolated from the head part, gut, silk gland, fat body, and epidermis of the *A. ventricosus* by using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from the *A. ventricosus* was 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 hybridization buffer containing 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. The 645 bp *A. ventricosus* SOD1 cDNA clone was labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA) for use as a probe for hybridization. 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 exposed to autoradiography film.

Results and Discussion

cDNA cloning, sequencing and phylogenetic analysis of AvSOD1

In a search of A. ventricosus ESTs, we identified a cDNA showing high homology with previously reported SOD1 genes. The cDNA clone including the full-length open reading frame (ORF) was sequenced and characterized. The nucleotide and its deduced amino acid sequences of the cDNA encoding SOD1 are presented in Fig. 1. The A. ventricosus SOD1 (AvSOD1) cDNA is 645 bp long, and contains an ORF of 498 nucleotides capable of encoding a 165 amino acid polypeptide with a predicted molecular mass of 17,114 Da and pI of 6.55. The ORF had both a start codon (ATG) and stop codon (TAA), indicating that the sequences contain the complete coding region. A putative polyadenylation signal, AATAAA, is located at nucleotide position 554-558.

Comparison of the deduced amino acid sequence of the AvSOD1 with other SOD1 sequences is shown in Fig. 2. The conserved residues in AvSOD1 are present at the corresponding positions of other SOD1s, representing that the regions are involved in the metal binding and catalysis. His56, 58, 73, and 130 theoretically function as cop-

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-53      gggacgcgtgttgctgtgtgtgcttttcagcgtggggtgggttagtggtcagc
  1      ATGCGGGCTTTCTGCAGTCCCCACGAAGTGGTTCCCAAGAGAGCCACCTGCACGATAAAT
  1      M  R  A  F  C  S  P  H  E  V  V  T  K  R  A  T  C  K  I  N
 61      AACGGGGACGTCCAAGGTGTCATCCAGTTGTATCAAGATCGTGTTACGGCTCCGGTGTCA
 21      N  G  D  V  Q  G  V  I  Q  L  Y  Q  D  R  V  K  A  P  V  S
121      GTGTCTGGCCAAATTAGAGGCTTGTCCCCTGGTCTTCACGGCTTCCACGTCCACCAATAT
 41      V  S  G  Q  I  R  G  L  S  P  G  L  H  G  F  H  V  H  Q  Y
181      GGTGATCTCTCAGGCGGGTGCGCTAGCGCTGGAGGGCATTTCAATCCATTTCAGAAAAAC
 61      G  D  L  S  G  G  C  A  S  A  G  G  H  F  N  P  F  Q  K  N
241      CATGGTGCTCCAACTGACGACGACAGACACGTGGGAGACTTGGGCAACATTGAGGCTGGA
 81      H  G  A  P  T  D  D  D  R  H  V  G  D  L  G  N  I  E  A  A
301      TCGGACGGAGTAGCTGCAATCAATATAGTCGATCACCAGCTGCGCCTCTGTGGACCCATC
101      S  D  G  V  A  A  I  N  I  V  D  H  Q  L  R  L  C  G  T  I
361      AGCGTCATGGGACGTGCCATTGTGGTTCACGCCCAACAGGACGATCTGGGCCGGGGCGGC
121      S  V  M  G  R  A  I  V  V  H  A  Q  Q  D  D  L  G  R  G  G
421      AACGAGGAGAGTAAGAAGACGGGCAACGCGGGCGCCCGGGTCGGGTGCTGCGTCATCGGA
141      N  E  E  S  K  K  T  G  N  A  G  A  R  V  G  C  C  V  I  G
481      GCAGCCAGCCTCCAATAAtcacattcttttttccccttttatttcacacttgtgacatattt
161      A  A  S  L  Q  ★
541      ttttcacatttccaataaagaagatgaataaaaaaaaaaaaaaaaaaaaa
```

Fig. 1. The nucleotide and deduced protein sequence of the AvSOD1 cDNA. The ATG start codon is boxed and the termination codon is indicated by asterisk. In the cDNA sequence, the polyadenylation sequence is double-underlined. The GenBank accession number is AY786991.

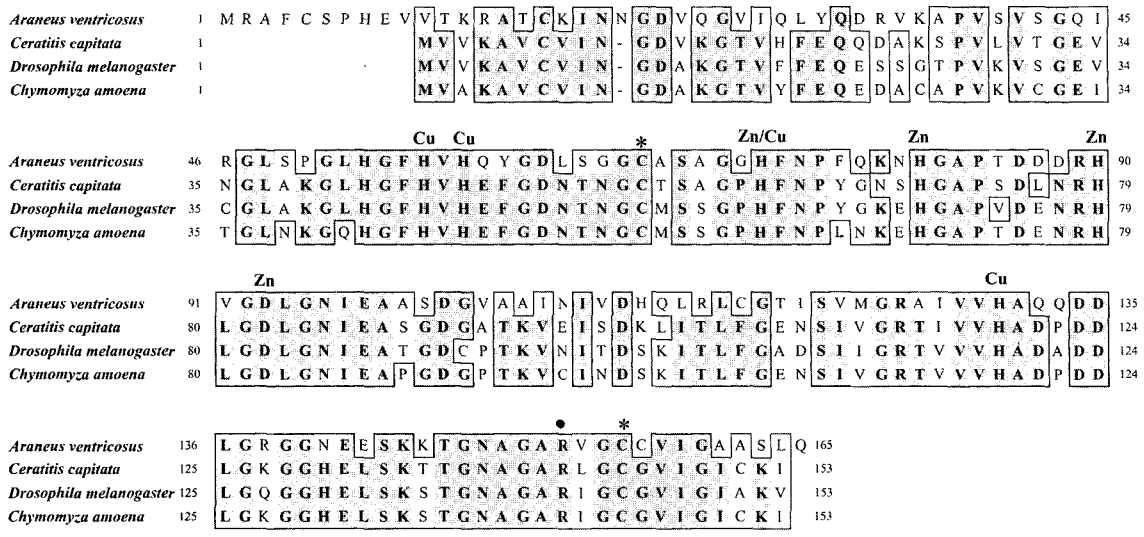
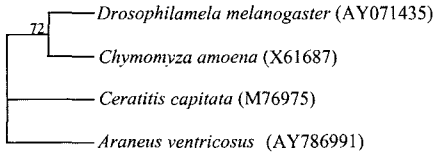


Fig. 2. Comparison of the deduced amino acid sequence of AvSOD1 with the known SOD1s. Invariant residues are shaded black. The SOD1 signature sequences are solid-boxed. Residues that form disulfide bridge (asterisk), ligate the metals (Cu or Zn), and guide the superoxide radical to the active site (solid circle) are also shown above the alignment. The sequence sources are described in Materials and Methods.

per binding sites, whereas His73, 81, 90 and Asp93 theoretically function as zinc binding sites. Cys67 and 156 are believed to be involved in disulfide bond formation, while Arg153 is believed to be necessary to guide the superoxide radical to the active site (Malinowski and Fridovich, 1979; Borders *et al.*, 1985; Parker *et al.*, 1986; Chaturvedi *et al.*, 2001; Parker *et al.*, 2004).

Among the known SOD1 sequences, the AvSOD1 showed 51% protein sequence identity to the Mediterranean fruit fly, *Ceratitis capitata* SOD1 (Kwiatowski *et al.*, 1992a), and 50% protein sequence identity to both *Drosophila melanogaster* SOD1 and *Chymomyza amoena* SOD1 (Kwiatowski *et al.*, 1992b) (Fig. 3).



	GenBank No.	Percent similarity 1	2	3	4
1. *Araneus ventricosus*	AY786991		69	68	66
2. *Ceratitis capitata*	M76975	51		87	88
3. *Drosophila melanogaster*	AY071435	50	78		90
4. *Chymomyza amoena*	X61687	50	81	83	

Percent identity

Fig. 3. Relationships among amino acid sequences of the AvSOD1 and the known SOD1s. (A) A maximum parsimony analysis for the amino acid sequences of AvSOD1 and known SOD1s. The sequence sources are described in the legend of Fig. 2. 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 similarities and identities of the deduced amino acid sequence of AvSOD1 among invertebrate SOD1 sequences.

SOD1 mRNA expression in *A. ventricosus* adult tissues

To characterize the expression of the AvSOD1 gene at the

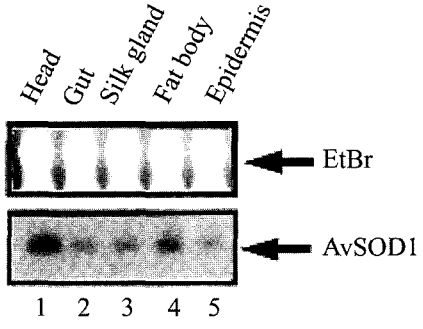


Fig. 4. AvSOD1 mRNA expression in *A. ventricosus* adult tissues. Total RNA was isolated from the head part (lane 1), gut (lane 2), silk gland (lane 3), fat body (lane 4), and epidermis (lane 5), respectively (upper panel). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis, transferred on to a nylon membrane, and hybridized with radiolabelled AvSOD1 cDNA probe (lower panel). Transcripts are indicated on the right side of the panel by an arrow.

transcriptional level, Northern blot analysis was performed using mRNA prepared from head part, gut, silk gland, fat body, and epidermis, respectively. Northern blot analysis showed that a hybridization signal was present in all tissues examined (Fig. 4). It is known that SOD1 is an abundant, largely cytosolic enzyme that scavenges superoxide anions (Crapo *et al.*, 1992; Sturtz *et al.*, 2001). The ubiquitous expression of the AvSOD1 supports the general observation that this protein may play an important role in the shielding of cells against oxidative damage.

In conclusion, we have cloned and characterized a novel cDNA encoding putative SOD1 from the spider *A. ventricosus*. We hope that the molecular characterization of SOD1 in *A. ventricosus* in this study will expand the understanding of invertebrate SOD1s.

Acknowledgements

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References

Borders, C. J., J. E. Saunders, D. M. Blech and I. Fridovich (1985) Essentially of the active site arginine residue for the normal catalytic activity of Cu,Zn superoxide dismutase. *Biochem. J.* **230**, 771-776.

Chary, P., D. Dillon, A. L. Schroeder and D. O. Natvig (1994) Superoxide dismutase (sod-1) null mutants of Neurospora crassa: oxidative stress sensitivity, spontaneous mutation rate and response to mutagens. *Genetics* **137**, 723-730.

Chaturvedi, S., A. J. Hamilton, P. Hobby, G. Zhu, C. V. Lowry and V. Chaturvedi (2001) Molecular cloning, phylogenetic analysis and three-dimensional modeling of Cu,Zn superoxide dismutase (CnSOD1) from three varieties of *Cryptococcus neoformans*. *Gene* **268**, 41-51.

Chung, E. H., K. S. Lee, J. H. Han, H. D. Sohn and B. R. Jin (2001) Analysis of expressed sequence tags of the spider, *Araneus ventricosus*. *Int. J. Indust. Entomol.* **3**, 191-199.

Crapo, J. D., T. Oury, C. Rabouille, J. W. Slot and L. Y. Chang (1992) Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. *Proc. Natl. Acad. Sci. USA* **89**, 10405-10409.

Dalton, T. P., H. G. Shertzer and A. Puga (1999) Regulation of gene expression by reactive oxygen. *Annu. Rev. Pharmacol. Toxicol.* **39**, 67-101.

Fabrizio, P., L. Liou, V. N. Moy, A. Diaspro, J. S. Valentine, E. B. Gralla and V. D. Longo (2003) SOD2 functions downstream of Sch9 to extend longevity in yeast. *Genetics* **163**, 35-46.

Fridovich, I. (1986) Superoxide dismutases. *Adv. Enzymol. Rel. Areas Mol. Biol.* **58**, 61-97.

Fridovich, I. (1995) Superoxide radical and superoxide dismutase. *Ann. Rev. Biochem.* **64**, 97-112.

Jamieson, D. J., S. L. Rivers and D. W. Stephen (1994) Analysis of Saccharomyces cerevisiae proteins induced by peroxide and superoxide stress. *Microbiology* **140**, 3277-3283.

Kwiatowski, J. M., D. Skarecky and F. J. Ayala (1992a) Structure and sequence of the Cu,Zn Sod gene in the Mediterranean fruit-fly *Ceratitis capitata*: Intron insertion/deletion and evolution of the gene. *Mol. Phylogenet. Evol.* **1**, 72-82.

Kwiatowski, J. M., D. Skarecky, M. Burgos and F. J. Ayala (1992b) Structure and sequence of the Cu,Zn superoxide dismutase gene of *Chymomyza amoena*: phylogeny of the genus and codon-use evolution. *Insect Mol. Biol.* **1**, 3-13.

Malinowski, D. P. and I. Fridovich (1979) Chemical modification of arginine at the active site of the bovine erythrocyte superoxide dismutase. *Biochemistry* **18**, 5909-5917.

McCord, J. M. and I. Fridovich (1969) The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. *J. Biol. Chem.* **244**, 6056-6063.

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

Mount, D. W. (1996) Reprogramming transcription. *Nature* **383**, 763-764.

Orr, W. C. and R. S. Sohol (1994) Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster. *Science* **263**, 1128-1130.

Parker, J. D., K. M. Parker, B. H. Sohol, R. S. Sohol and L. Keller (2004) Decreased expression of Cu-Zn superoxide dismutase 1 in ants with extreme lifespan. *Proc. Natl. Acad. Sci. USA* **101**, 3486-4389.

Parker, M. W., F. Bossa, D. Barra, W. H. Bannister and J. V. Bannister (1986) Structural and evolutionary relationships between the eukaryotic superoxide dismutase; in *Superoxide and superoxide dismutase in chemistry, biology and medicine*. Rotilio, G. (ed.), pp. 237-245, Elsevier Sci. Publ., Amsterdam.

Parkes, T. L., A. J. Elia, D. Dickinson, A. J. Hilliker, J. P. Phillips and G. L. Boulianne (1998) Extension of *Drosophila lifespan* by overexpression of human SOD1 in motorneurons. *Nat. Genet.* **19**, 171-174.

Sohol, R. S., A. Agarwal, S. Agarwal and W. C. Orr (1995) Simultaneous overexpression of copper- and zinc-containing superoxide dismutase and catalase retards age-related oxidative damage and increases metabolic potential in *Drosophila melanogaster*. *J. Biol. Chem.* **270**, 15671-15674.

Sturtz, L. A., K. Diekert, L. T. Jensen, R. Lill and V. C. Culotta (2001) A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. *J. Biol. Chem.* **276**, 38084-38089.

Sun, J. and J. Tower (1999) FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression

can extend the life span of adult *Drosophila melanogaster flies. Mol. Cell. Biol.* **19**, 216-228.

Swofford, D. L. (2000) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0. Sinauer Sunderland. MA.

Tainer, J. A., E. D. Getzoff, K. M. Beem, J. S. Richardson and D. C. Richardson (1982) Determination and analysis of the 2 Å structure of copper, zinc superoxide dismutase. *J. Mol. Biol.* **160**, 181-217.