Plant Physiol. (1991) 96, 1391-1392
0032-0889/91/96/1391/02/$01.00/0

Received for publication March 22, 1991
Accepted May 12, 1991

Plant Gene Register

Nucleotide Sequence of a Complementary DNA Encoding Pea Cytosolic Copper/Zinc Superoxide Dismutase[1]

Deborah A. White and Barbara A. Zilinskas*

Department of Biochemistry and Microbiology, Lipman Hall, Cook College, Rutgers University, New Brunswick, New Jersey 08903-0231

SOD[2] (EC 1.15.1.1), a metalloenzyme that catalyzes the disproportionation of superoxide radicals to molecular oxygen and hydrogen peroxide, is important in cellular defense against oxygen toxicity (reviewed in ref. 5). In pea (*Pisum sativum* L.) leaf, three forms of the enzyme exist, all of which are nuclear encoded and catalyze the same dismutation reaction. The Cu/Zn SOD is the predominant form of the enzyme, with one isozyme found in the chloroplast stroma and the other in the cytosol. The Mn SOD is localized in mitochondria and has been reported by one group (2) to be also found in peroxisomes.

Our laboratory was the first to clone and characterize a cDNA encoding the chloroplastic Cu/Zn SOD (10). A second cDNA encoding the same pea enzyme was recently published (6). We now report the nucleotide sequence of the cytosolic Cu/Zn SOD cloned from a λgt11 cDNA library (3) constructed from mRNA extracted from leaves of 7- to 10-d pea seedlings (Table I, Fig. 1). The clone was isolated using a 22-base synthetic oligonucleotide complementary to the amino acid sequence CGIIGLQG. This sequence, found at the protein's carboxy terminus, is highly conserved among plant cytosolic Cu/Zn SODs but not chloroplastic Cu/Zn SODs. The 738-base pair sequence contains an open reading frame specifying 152 codons and a predicted M_r of 18,024 D. The deduced amino acid sequence is highly homologous (79–82% identity) with the sequences of other known plant cytosolic Cu/Zn SODs (1, 8, 9, 11) but less highly conserved (63–65%) when compared with several chloroplastic Cu/Zn SODs (7, 8, 12), including pea (10).

ACKNOWLEDGMENT

We gratefully acknowledge Dr. J. Stephen Gantt for providing us with the pea leaf λgt11 cDNA library.

Table I. *Characteristics of a cDNA Encoding Pea Cytosolic Cu/Zn SOD*

Organism:
Pisum sativum L., cv 'Little Marvel.'
Location in Genome:
Nuclear genome; chromosome location not known.
Gene, Function, Pathway:
Sod 2; cytosolic Cu/Zn SOD (EC 1.15.1.1); dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen.
Techniques:
cDNA cloning; double-stranded plasmid sequencing; subcloning using useful restriction sites and oligonucleotide primers; complete dideoxy sequencing of both strands.
Method of Isolation, Subsequent Identification:
Hybridization with degenerate 22b oligonucleotide mixture based on a conserved 8-amino acid carboxy-terminal sequence, designed from three known plant cytosolic Cu/Zn SODs. Sequence comparison with published cytosolic Cu/Zn SODs including cabbage (11), maize (1), tomato (8), and spinach (7) of 78.8, 81.5, 81.5, and 81.6% identity, respectively.
Expression Characteristics:
Transcript of 780 nucleotides; steady-state level of the transcript is very low in leaves of 10-d-old pea seedlings. Transcript termini not determined.
Features of the cDNA Structure:
Translation start site at nucleotide 16 and stop site at nucleotide 471.
Codon Usage:
A strong bias for T in the third codon position; codons not used include ACG(T), TGG(W), TAT(Y), TAC(Y), TTA(L), TTT(F), TCT(S), CGN(R), CTG(L), CTA(L), CCG(P), CCC(P).
(G + C) Content:
43% (47% in the coding region).
Structural Features of the Protein:
Open reading frame of 152 amino acids, M_r 18,042 D. There are many invariant residues that are involved in maintaining the active site (15 residues), dimer interface (4 residues), and β-barrel (4 residues) (see ref. 4); of these, we note ligands to Cu (His-45, -47, -62, -70, -79, and -119) and Zn (Asp-83).
Antibodies:
Raised in rabbit against pea cytosolic Cu/Zn SOD (9).
Subcellular Location:
Cytosol.
EMBL Accession No:
M63003.

---

[1] This is New Jersey Agricultural Experiment Station Publication No. D-01905-2-91, supported in part by state funds and by the U. S. Hatch Act. This work is also supported by the Cooperative State Research Service, U. S. Department of Agriculture, under Agreement No. 89-3471-4502.

[2] Abbreviation: SOD, superoxide dismutase.

```
         .         .         .         .         .         .
        10                  30                  50
GGATCACATTGAACAATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCG
               M  V  K  A  V  A  V  L  S  N  S  N  E  V  S
         .         .         .         .         .         .
        70                  90                 110
GGTACTATTAACTTCAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCT
G  T  I  N  F  S  Q  E  G  N  G  P  T  T  V  T  G  T  L  A
         .         .         .         .         .         .
       130                 150                 170
GGTCTTAAGCCTGGCCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGT
G  L  K  P  G  L  H  G  F  H  I  H  A  L  G  D  T  T  N  G
         .         .         .         .         .         .
       190                 210                 230
TGCATTTCAACTGGACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGAT
C  I  S  T  G  P  H  F  N  P  N  G  K  E  H  G  A  P  E  D
         .         .         .         .         .         .
       250                 270                 290
GAGACTAGACATGCTGGTGATCTTGGAAATATCAATGTTGGTGATGATGGAACTGTAAGC
E  T  R  H  A  G  D  L  G  N  I  N  V  G  D  D  G  T  V  S
         .         .         .         .         .         .
       310                 330                 350
TTCACCATTACTGACAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCT
F  T  I  T  D  N  H  I  P  L  T  G  T  N  S  I  I  G  R  A
         .         .         .         .         .         .
       370                 390                 410
GTTGTTGTCCATGCCGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACT
V  V  V  H  A  D  P  D  D  L  G  K  G  G  H  E  L  S  K  T
         .         .         .         .         .         .
       430                 450                 470
ACTGGAAATGCTGGTGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACT
T  G  N  A  G  G  R  V  A  C  G  I  I  G  L  Q  G
         .         .         .         .         .         .
       490                 510                 530
ACTCTCCACTGTGCGTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTT
         .         .         .         .         .         .
       550                 570                 590
GCTTGTTTAGGGTCTGATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTC
         .         .         .         .         .         .
       610                 630                 650
AATGGCTATATGACTGCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGT
         .         .         .         .         .         .
       670                 690                 710
TGTCATGCTTGTTTTCAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAAT
         .
       730
AAAAAAAAAAAAAAAAAA
```

Figure 1. Nucleotide sequence and deduced amino acid sequence of a cDNA encoding pea cytosolic Cu/Zn SOD.

LITERATURE CITED

1. **Cannon RE, White JA, Scandalios JG** (1987) Cloning of cDNA for maize superoxide dismutase 2 (SOD2). Proc Natl Acad Sci USA **84:** 179–183
2. **del Rio LA, Lyon DS, Olah I, Glick B, Salin ML** (1983) Immunocytochemical evidence for a peroxisomal localization of manganese superoxide dismutase in leaf protoplasts from a higher plant. Planta **158:** 216–224
3. **Gantt JS, Key JL** (1986) Isolation of nuclear encoded plastid ribosomal protein cDNAs. Mol Gen Genet **202:** 186–193
4. **Getzoff ED, Tainer JA, Stempien MM, Bell GI, Hallewell RA** (1989) Evolution of CuZn superoxide dismutase and the Greek key β-barrel structural motif. Proteins Struct Funct Genet **5:** 322–336
5. **Hassan HM, Scandalios JG** (1990) Superoxide dismutase in aerobic organisms. *In* RG Alsher, JR Cumming, eds, Plant Biology, Vol 12, Wiley-Liss, New York, pp 175–200
6. **Isin SH, Burke JJ, Allen RD** (1990) Sequence divergence of pea Cu/Zn superoxide dismutase II cDNAs. Plant Mol Biol **15:** 789–791
7. **Kitagawa Y, Tsunasawa S, Tanaka N, Katsuke Y, Sukiyama F, Asada K** (1986) Amino acid sequence of copper, zinc-superoxide dismutase from spinach leaves. J Biochem **99:** 1289–1298
8. **Perl-Treves R, Nacmias B, Aviv D, Zeelon EP, Galun E** (1988) Isolation of two cDNA clones from tomato containing two different superoxide dismutase sequences. Plant Mol Biol **11:** 609–623
9. **Sakamoto A, Ohsuga H, Wakaura M, Mitsukawa N, Hibino T, Sasaki Y, Tanaka K** (1990) Nucleotide sequence of cDNA for the cytosolic Cu/Zn-superoxide dismutase from spinach (*Spinacia oleracea L*). Nucleic Acids Res **18:** 4923
10. **Scioli JR, Zilinskas BA** (1988) Cloning and characterization of a cDNA encoding the chloroplastic copper zinc-superoxide dismutase from pea. Proc Natl Acad Sci USA **85:** 7661–7665
11. **Steffens GJ, Michelson AM, Otting F, Puget K, Strassburger W, Floché L** (1986) Primary structure of Cu-Zn superoxide dismutase of *Brassica oleracea* proves homology with corresponding enzymes of animals, fungi and prokaryotes. Biol Chem Hoppe-Seyler **367:** 1007–1016
12. **Tepperman CK, Dunsmuir P** (1988) Cloning and nucleotide sequence of petunia gene encoding a chloroplast-localized superoxide dismutase. Plant Mol Biol **11:** 871–872