Molecular Cloning of a cDNA Encoding Copper/Zinc Superoxide Dismutase from Papaya Fruit and Overexpression in *Escherichia coli*

Ming-Tse Lin,[†] Ti-Jung Kuo,[‡] and Chi-Tsai Lin*[,‡]

Department of Bioengineering, Tatung Institute of Technology, Taipei, Taiwan, and Institute of Marine Biotechnology, National Taiwan Ocean University, Keelung, Taiwan

A full-length complementary DNA (cDNA) clone encoding a putative copper/zinc superoxide dismutase (Cu/Zn SOD) of papaya fruit, *Carica papaya* L. cv. Tainong 2, was amplified according to the polymerase chain reaction technique from cDNA synthesized from fruit messenger RNA. Nucleotide sequence analysis of this cDNA clone revealed that it comprised a complete open reading frame coding for 152 amino acid residues. The deduced amino acid sequence showed higher identity (81.6−84.9%) with the sequences of the cytosolic Cu/Zn SODs than those of the chloroplast from other plant species, and no recognizable plastid targeting peptide was found. These suggest that the papaya fruit cDNA clone encodes a cytosolic Cu/Zn SOD. The residues required for coordinating copper (His-45, -47, -62, and -119) and zinc (His-62, -70, and -79 and Asp-82), as well as the two cysteines (56 and 145) that form a single disulfide bond, are conserved as they are among all reported Cu/Zn SOD sequences. In addition, the coding region of Cu/Zn SOD cDNA from papaya was introduced into an expression vetor, pET-20b(+), and transformed into *Escherichia coli* AD494-(DE3)pLysS. A predominant protein band was detected by Coomassie blue staining of native PAGE, and activity staining confirmed the result of Coomassie blue staining. These indicate that this Cu/Zn SOD cDNA clone can overexpress active Cu/Zn SOD enzyme in *E. coli*.

Keywords: *Copper/zinc superoxide dismutase; Cu/Zn SOD; papaya (Carica papaya L. cv. Tainong 2)*

INTRODUCTION

Superoxide dismutases (SOD; superoxide:superoxide oxidoredutase, EC 1.15.1.1) are enzymes that dismutate molecular oxygen radicals and thus prevent the lethal effects of oxygen radicals in aerobic organisms. SODs are metalloproteins and are classified into three types (Mn, Fe, and Cu/Zn SOD) depending on the metal found in the active site (Brock et al., 1980; Fridovich, 1986; Harris et al., 1980).

In plants, the activity of SOD increases in response to a variety of environmental and chemical stimuli (Fridovich, 1986; Perl-Treves and Galun, 1991). In transgenic plants, alfalfa overexpressing Mn SOD tended to reduce injury from water deficit stress and improve tolerance of oxidative stress in adaptation to field environments (McKersie et al., 1996). The most prominent plant SOD is a Cu/Zn isoenzyme found in the cytosol and plastids (Sakamoto et al., 1992). Many plant Cu/Zn SOD complementary DNA (cDNA) from leaves or seedlings have been sequenced and compared, but there are no reports on Cu/Zn SOD cDNA from fruit tissue.

Previously, we have cloned and sequenced the cDNA of Cu/Zn SOD from sweet potato tuberous root (Lin et al., 1993) and analyzed its gene structure (Lin et al., 1995a). The subunit interaction enhancing the enzyme activity and stability has been established (Lin et al., 1995b), and Arg-141 to Ser in sweet potato Cu/Zn SOD by site-directed mutagenesis showed unusual thermal stability (Lin et al., 1996). We also have cloned and sequenced a Mn SOD from sweet potato callus induced from leaves (Lin et al., 1997). Here we report the cDNA sequence and deduced amino acid sequence of Cu/Zn SOD from papaya fruit. The coding region of Cu/Zn SOD cDNA was introduced into an expression vector, pET-20b(+), and transformed into *Escherichia coli* AD494(DE3)pLysS. It was demonstrated that this cDNA clone can overexpress an active Cu/Zn SOD in *E. coli*.

MATERIALS AND METHODS

Materials. Papaya (*Carica papaya* L. cv. Tainong 2) fruit with color-break appearance was harvested from a local papaya orchard. The fruit was stored frozen at −70 °C until used.

mRNA Preparation and cDNA Synthesis. Thirty grams of frozen papaya tissue was mixed with 2 g of glass beads and ground to powder in a ceramic mortar with liquid nitrogen. The sample was dissolved in 80 mL of extraction buffer containing 57.4 g of guanidium hydrochloride (Boehringer Mannheim Biochemical), 0.4 g of sodium sarcosyl (Serva), 2 mL of 1 M sodium citrate (Sigma Chemical) (pH 7.0), and 560 μL of 2-mercaptoethanol (Sigma Chemical). Total RNA was prepared by using the guanidium hydrochloride procedure (Yeh et al., 1991). The poly(A)$^+$ RNA was isolated according to the oligo-(dT) cellulose (Boehringer Mannheim Biochemical) chromatography (Aviv and Leder, 1972). Double-strand blunted cDNA was synthesized using a kit (cDNA synthesis module RPN1256) from Amersham (Little Chalfont, Buckinghamshire, England).

* Address correspondence to this author at the Institute of Marine Biotechnology, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 2024, Taiwan (telephone 886-2-4622192, ext. 5514; fax 886-2-4622320; e-mail ctlin@ntou66.ntou.edu.tw).

[†] Tatung Institute of Technology.

[‡] Institute of Marine Biotechnology.

S0021-8561(97)00684-5 CCC: $15.00 © 1998 American Chemical Society
Published on Web 01/19/1998

```
   1                                                    AGGTCTCTCCCCCTACTTGTTTATCGTACGGGTGTTCTGAGATCACATACAAAA

  55  ATG GTG AAG GCT GTA GCT GTC CTT AGC AGC AGT GAG GGT GTC AGT GGA ACT ATC TTC TTC
   1   M   V   K   A   V   A   V   L   S   S   S   E   G   V   S   G   T   I   F   F

 115  ACC CAA GCA GCA GAT GGC CCA ACC ACC GTA ACT GGT GAA ATT TCT GGC CTA AAG CCG GGG
  21   T   Q   A   A   D   G   P   T   T   V   T   G   E   I   S   G   L   K   P   G

 175  CAT CAC GGC TTC CAT GTT CAT GCC TTG GGT GAC ACA ACA AAT GGC TGC ATG TCG ACT GGT
  41   H   H   G   F   H   V   H   A   L   G   D   T   T   N   G   C   M   S   T   G

 235  CCT CAT TTC AAT CCT GCT GGC AAA GAA CAT GGT GCT CCA GAG GAT GAC ATC CGT CAT GCT
  61   P   H   F   N   P   A   G   K   E   H   G   A   P   E   D   D   I   R   H   A

 295  GGT GAC CTT GGA AAT GTA AAT GTT GGT GAT GAT GGC AAA GTT AGC TTC TCA ATT ATC GAC
  81   G   D   L   G   N   V   N   V   G   D   D   G   K   V   S   F   S   I   I   D

 355  AGT CAG ATT CCT CTT ACT GGA CCA AAC TCC ATT GTT GGA AGG GCT GTT GTT GTC CAC GCT
 101   S   Q   I   P   L   T   G   P   N   S   I   V   G   R   A   V   V   V   H   A

 415  GAT CCT GAT GAT CTT GGC AAA GGG GGG CAT GAG CTC AGT AAG ACT ACT GGA AAT GCT GGG
 121   D   P   D   D   L   G   K   G   G   H   E   L   S   K   T   T   G   N   A   G

 475  GGC AGA GTT GCT TGT GGA GTC ATC GGT CTC CAA GGT TGA AGAATCATTTCCAGAAATATTAGAATC
 141   G   R   V   A   C   G   V   I   G   L   Q   G   *

 541  AGTTGAAAAGCCCGCGGATCACATGCTGCCTTACCGGTTTTTGGCAGTAAACTCGGTGACTAGAAACTCTTTGAGTAAA

 620  TGGAGATTACTTGTTTGTGTATTTTGGGTTAGACTGGTTTTTGTGGTCTTAAAATTGTGAGTTGTTCTTGTTATTGCTC

 699  TGAAATAGCTCTCCTCTTATCAGCATTATATCATCATCCCCTGAAATTTTCATGCCCCCAAAAAAAAAAA     ←768
```

Figure 1. Nucleotide sequence of a papaya Cu/Zn SOD cDNA and the deduced amino acid sequence. Numbers to the left refer to nucleotide and its deduced amino acid residues. Consensus sequence of the translation start site is underlined, and the polyadenylation signal is marked by a double underline. The asterisk denotes the translation stop signal.

Subcloning and DNA Sequence Analysis. One microgram of blunted cDNA was ligated with 30 pmol of Marathon cDNA adaptor (Clontech, Palo Alto, CA) at 22 °C for 4 h. A Ta−N primer (5′ TTA CCA AGA TCA TCG GGA TC 3′) was synthesized according to the sequence of sweet potato Cu/Zn SOD (Lin et al., 1993). Using 0.1 μg of the ligated cDNA as a template, 10 pmol of Clontech adaptor primer and 10 pmol of Ta−N primer were added. One 0.4 kbp DNA (5′-RACE:5′-cDNA end) was amplified by using the PCR technique. The 0.4 kbp fragment was subcloned into pGEM-T (Promega, Madison, WI) using JM109 as a host. Nucleotide sequence was determined in both directions according to the dideoxy technique with a Taq track sequencing systems kit (Promega). A PaCu-4 primer (5′ ATC CGT CAT GCT GGT GAC CTT GGA 3′) was synthesized according to the determined 0.4 kbp DNA (5′-RACE) sequence. Again using 0.1 μg of the above ligated cDNA as a template, one 0.35 kbp DNA (3′-RACE: 3′-cDNA end) was amplified by PCR with adaptor primer and PaCu-4 primer. The 0.35 kbp DNA fragment was subcloned and sequenced. Sequence analysis revealed that 5′-RACE and 3′-RACE cover the full-length Cu/Zn SOD cDNA. Using the 0.4 kbp DNA (5′-RACE) and the 0.35 kbp DNA (3′-RACE) as templates, one fused full-length cDNA of Cu/Zn SOD was created by PCR technique (EMBL accession no. is Y13610).

Recombinant DNA Preparation and Transformation. Using 100 ng of blunted cDNA as a template, 10 pmol of each 5′-primer and 3′-primer was added (5′-primer, 5′ CCC ATG GTG AAG GCT GTA GCT GTC 3′; 3′-primer, 5′ GGA ATT CCC TTG GAG ACC GAT GAC TCC 3′). A 0.45 kbp DNA fragment, amplified by PCR technique, was ligated with pGEM-T (Promega) and transformed into *E. coli* JM109 host. A positive clone was selected by hybridization with ^{32}P-labeled Cu/Zn SOD cDNA as probe. After the plasmid DNA of positive clone was isolated and digested with *Nco*I and *Eco*RI, it was separated by 0.8% agarose gel electrophoresis. A 0.45 kbp insert DNA containing *Nco*I and *Eco*RI sites was recovered from the gel and ligated with pET-20b(+) (pretreated with *Nco*I and *Eco*RI) from Novagen (Madison, WI) and transformed into *E. coli* AD494(DE3)pLysS as host. The transformed clone was selected by hybridization with ^{32}P-labeled Cu/Zn SOD cDNA as probe.

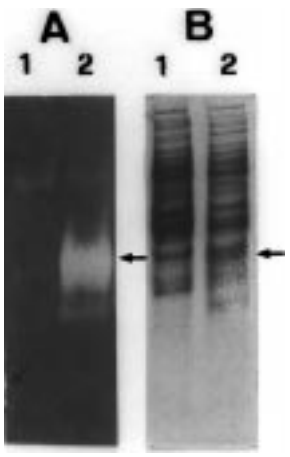

Figure 2. Activity (A) and Coomassie blue (B) staining of the crude extract of recombinant papaya Cu/Zn SOD: lane 1, AD494(DE3)pLysS carrying pET-20b(+) as control; lane 2, AD494(DE3)pLysS carrying recombinant Cu/Zn SOD cDNA. An arrow denotes Cu/Zn SOD activity (panel A) and the corresponding protein band (panel B), respectively.

Culture and Enzyme Production. Transformed *E. coli* cells were grown at 37 °C in 3 mL of Luria Bertani medium containing 50 μg/mL ampicillin (Sigma Chemical), 30 μg/mL kanamycin (Gibco BRL), and 34 μg/mL chloramphenicol (Gibco BRL) until A_{600} reached 0.9. Isopropyl β-D-thiogalactopyranoside (IPTG; Gibco BRL) was added to a concentration of 1 mM. The culture was incubated at 37 °C for 5 h on a rotary shaker (120 rpm), and the bacterial cells were harvested by centrifugation at 9000*g* for 1 min. The cells were suspended in 0.2 mL of Tris buffer (10 mM Tris buffer, pH 7.5, containing 5% glycerol); 0.1 g of glass bead was added and vortexed for 5 min and then centrifuged at 13000*g* for 5 min. The supernatant fraction was subjected to enzyme assay.

```
Cpsodc
Sosodc
Ibsodc
Slsodc
Pssodc
Atsodc
Zmsodc
Ossodc
Sosodp  MAAHTILASA PSHTTFSLIS PFSSTPTNAL SSSLQSSSFN GLSFKLSPTT    50
Pssodp  ..SQ.LVSP- ----------. .L..H----- --..LRT.FS .V.V..AP--    31
Slsodp  ....S.FTTT S--..N.FLY .I..SS---- ..PNIN...L .V.LNVNAKF    44

Cpsodc                        MVKAVAVLS SSEGVSGTIF FTQAADGPTT    29
Sosodc                        .G...V... ........VY .A.EG.....    29
Ibsodc                        ......... .......... .S.EG.....    29
Slsodc                        ........N ........YL ...VGVA...    29
Pssodc                        ......... N.NE.....N .S.EGN....    29
Atsodc                        .A.G....N .....T.... ...EG..V..    29
Zmsodc                        ........A GTD-.K.... .S.EG.....    28
Ossodc                        ........A .....K.... .S.EG....S    29
Sosodp  QSLSLSTSAA S--KPLTIVA ATK......K GTSN.E.VVT L..ED.....    98
Pssodp  ---QF..L.T .NF....V.. .A....S..K GTSA.E.VVT L..DDE....    78
Slsodp  -GQ..TLY.V TTP....VF. .TK......K GNSN.E.VVT LS.DD.....    93

                        * *                 *       *       *
Cpsodc  VTGEISGLKP GHHGFHVHAL GDTTNGCMST GPHFNPAGKE HGAPEDDIRH    79
Sosodc  ...NV..... .L........ .......... ...Y..N... .......V..    79
Ibsodc  ...NV..... .L........ .......... .......... ....G..N..    79
Slsodc  .N.N...... .L........ .......... ...Y...... ......EV..    79
Pssodc  ...TLA.... .L....I... .......I.. ......N... ......ET..    79
Atsodc  .S.TV..... .L........ .......... ......D..T ......AN..    79
Zmsodc  ...S...... .L........ .......... ......V... ......ED..    78
Ossodc  ...SV..... .L........ .......... ......T... ....Q.EN..    79
Sosodp  .NVR....A. .K....L.EF .......... ......DK.T ......EV..   148
Pssodp  .NVR.T..T. .L....L.EY .......I.. ......NKLT ......E...   128
Slsodp  .NVR.T..A. .L....L.EY .......... .A....NKLT ....G.E...   143

          *                                         *
Cpsodc  AGDLGNVNVG DDGKVSFSII DSQIPLTGPN SIVGRAVVVH ADPDDLGKGG   129
Sosodc  ......IT.. ...TAT.T.. ......S... .......... .E.....R..   129
Ibsodc  ......IT.. E..TA..T.T .K......A. .VI....... G.........   129
Slsodc  ......IT.. E..TA..T.T .K.......Q ..I....... ..........   129
Pssodc  ......I... ...T...T.T .NH.....T. ..I....... ..........   129
Atsodc  ......IT.. ...TAT.T.T .C........ .......... ..........   129
Zmsodc  .......TA. E..V.NVN.T ......A..H ..I....... ..........   128
Ossodc  ......ITA. A..VANVNVS ........A. ..I....... ..........   129
Sosodp  ......IVAN T..VAEAT.V .N........ .V....L... ELE.......   198
Pssodp  ......IVAN AE.VAEAT.V .N........ .V....L... ELQ.......   178
Slsodp  ......IVAN A..VAEVTLV .N........ .V....L... ELE.......   193

Cpsodc  HELSKTTGNA GGRVACGVIG LQG                                152
Sosodc  .......... .......I.. ...                                152
Ibsodc  .....S.... .......I.. ...                                152
Slsodc  .....S.... ...I...I.. ...                                152
Pssodc  .......... .......I.. ...                                152
Atsodc  ....LA.... .......I.. ...                                152
Zmsodc  .....S.... .......I.. ...                                151
Ossodc  .......... .......I.. ...                                152
Sosodp  ....P..... ...L....V. .TPV                               222
Pssodp  ....LS.... ...L....V. .TPV                               202
Slsodp  ....L..... ...L....V. .TPI                               217
```

Figure 3. Optimal alignment of Cu/Zn SOD among several plant species. The alignment of amino acid sequences translated from cDNA sequences used the PILEUP program in Wisconsin Sequence Analyze Package. The accession numbers in EMBL and the names for these sequences are as follows: Cpsodc, papaya, this study (EMBL no. Y13610); Sosodc, spinach (X53872); Ibsodc, sweet potato (X73139); Slsodc, tomato (X14040); Pssodc, pea (M63003); Atsodc, *Arabidopsis* (X60935); Zmsodc, maize (M15175); Ossodc, rice (D01000); Sosodp, spinach (D10244); Pssodp, pea (J04087); Slsodp, tomato (X14041). Numbers refer to amino acid residues of each species. A dot refers to identities with papaya, and a dash denotes deletion. Residues coordinating copper and zinc are indicated with asterisks. The two cycteines that form a disulfide bridge are underlined.

Enzyme Assay by Activity Staining on Native PAGE. Two 10 μL equivalents of the enzyme extraction were electrophoresed in 10% native acrylamide gel for 3 h at 100 V, and the gel was cut into two parts: one was assayed for Cu/Zn SOD activity staining as described previously (Beauchamp and Fridovich, 1971), and the other part was stained with Coomassie blue.

RESULTS AND DISCUSSION

Figure 1 shows the nucleotide and deduced amino acid sequences of a Cu/Zn SOD cDNA clone. Sequence analysis showed that the cDNA was full length, containing a complete open reading frame coding for 152 amino acid residues. The translated protein does not

Table 1. Comparison (Percent Identity, Percent Similarity) of Amino Acid Sequences for Cu/Zn SOD of Papaya Fruit and Other Organisms

type	source	English plant name	EMBL no.	organ	cultivar	% identity	% similarity
cytoplasmic	*Carica papaya*	papaya	Y13610	fruit	Tainong 2		
	Spinacia oleracea L	spinach	X53872	leaf	King of Denmark	84.967	92.157
	Ipomoea batatas	sweet potato	X73139	tuber root	Tainong 57	85.621	92.810
	Solanum lycopersicum	tomato	X14040	seedling leaf	Sherry-type	83.007	90.850
	Pisum sativum	pea	M63003	seedling leaf	Little Marvel	83.007	90.850
	Arabidopsis thaliana	thale cress	X60935	leaf, stem	Columbia	83.007	86.928
	Zea mays	maize	M15175			82.895	88.158
	Oryza sativa	rice	D01000	developing seed	Nipponhare	81.699	88.235
plastidic	*Spinacia oleracea* L	spinach	D10244	seedling leaf		67.320	75.817
	Pisum sativum	pea	J04087	seedling leaf	Progress No. 9	63.399	73.203
	Solanum lycopersicum	tomato	X14041	seedling leaf	Sherry-type	64.052	73.203

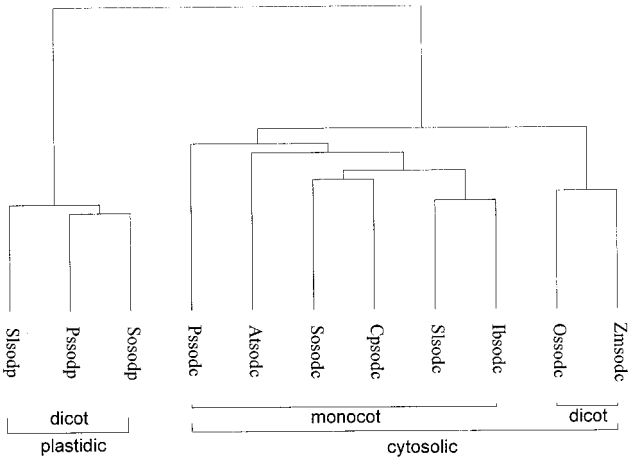

Figure 4. Phylogenetic analysis of Cu/Zn SOD based on amino acid sequences from various plants. The transit peptides for chloroplastic sequences were removed before the alignment. The alignments of the sequences created using the PILEUP program in Figure 3 were used for the phylogenetic analysis.

contain a transit peptide, suggesting that the enzyme is cytosolic as reported from other sources (Kanematsu and Asada, 1990). The DNA sequence translation start site (A AAA ATG G) fully matches the consensus sequence (A ACA ATG G) reported in plants (Lütcke et al., 1987).

The coding region was amplified and ligated with an expression vector, pET-20b(+), and transfomed into *E. coli* for expression. Figure 2 shows the predominant protein was detected by Coomassie blue staining of 10% native PAGE (panel B, an arrow denotes Cu/Zn SOD protein), and activity staining (panel A, an arrow denotes Cu/Zn SOD activity) confirmed the result of Coomassie blue staining.

Figure 3 shows that seven residues coordinating copper (His-45, -47, -62, and -119) and zinc (His-62, -70, and -79 and Asp-82), as well as the two cysteines (56 and 145) that form a single disulfide bond, are conserved as they are among all reported Cu/Zn SOD sequences (Fridovich, 1986). Table 1 shows higher identity (81.6−84.9%) with the amino acid sequence of the cytosolic Cu/Zn SOD than that of the other chloroplast from other plant species. These comparisons were done by the program of the University of Wisconsin Genetics Computer Group.

From the alignment of published Cu/Zn SOD protein sequences, we constructed a phylogenetic tree (Figure 4) which suggests that plant Cu/Zn SODs are divided into two groups according to their cytoplasmic (lacking plastidic targeting sequence) or chloroplastic (containing targeting sequence) location (Bordo et al., 1994). However, the two Cu/Zn isozymes arise from the same ancestor gene in the nucleus, and the gene, for the preexisting duplicated cytosolic enzyme, obtained a transit peptide sequence allowing it to be rerouted into the plastid or other organelles (Tanaka et al., 1996; Kanematsu and Asada, 1990). Therefore, the cytosolic Cu/Zn SODs have been evolving rapidly in recent times within the past 100 million years (Smith and Doolittle, 1992). In addition, several isozymes of Cu/Zn SOD are found in cytosol of most green plants, while only a single or no Cu/Zn SOD is found in the chloroplast (Baum et al., 1983; Kanematsu and Asada, 1989a,b, 1990; Sen Gupta et al., 1993; Streller et al., 1994) and it has a more constant evolution rate (Karpinski et al., 1992; Oseki et al., 1989). The increase in evolution rate and gene duplication of cytosolic Cu/Zn SOD may be to improve the adaptation for some general environmental change (Sakamoto et al., 1995; Smith and Doolittle, 1992).

CONCLUSION

A full-length cDNA encoding a putative Cu/Zn SOD from the papaya fruit was amplified according to the PCR technique. This clone comprises a complete open reading frame coding for 152 amino acid residues. The coding region was introduced into an expression vector, pET-20b(+), and transformed into *E. coli* AD494(DE3)-pLysS. The expression of the recombinant Cu/Zn SOD cDNA was confirmed by enzyme activity staining on native acrylamide gel.

LITERATURE CITED

Aviv, H.; Leder, P. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1408−1412.

Baum, J. A.; Chandlee, J. M.; Scandalios, J. G. Purification and partial characterization of a genetically defined superoxide dismutase (SOD-1) associated with maize chloroplasts. *Plant Physiol.* **1983**, *73*, 31−35.

Beauchamp, C.; Fridovich, I. Improved assays and an assay applicable to acrylamide gel. *Anal. Biochem.* **1971**, *44*, 276−287.

Bordo, D.; Djinovic, K.; Bolognesi, M. Conserved patterns in the Cu,Zn superoxide dismutase family. *J. Mol. Biol.* **1994**, *1238*, 366−386.

Brock, C. J.; Walker, J. E. Superoxide dismutase from *Bacillus stearothermophilus.* Complete amino acid sequence of a manganese enzyme. *Biochemistry* **1980**, *19*, 2873−2882.

Fridovich, I. Superoxide dismutases. *Adv. Enzymol.* **1986**, *58*, 61−97.

Harris, J. I.; Auffret, A. D.; Northrop, F. D.; Walker, J. E. Structural comparisons of superoxide dismutases. *Eur. J. Biochem.* **1980**, *106*, 297−303.

Kanematsu, S.; Asada, K. CuZn-superoxide dismutases in rice: occurrence of an active, monomeric enzyme and two types of isozyme in leaf and non-photosynthetic tissue. *Plant Cell Physiol*. **1989a**, *30*, 381−391.

Kanematsu, S.; Asada, K. CuZn-superoxide dismutases from the fern *Equisetum arvense* and the green alga *Spirogyra* sp.: occurrence of chloroplast and cytosol types of enzyme. *Plant Cell Physiol*. **1989b**, *30*, 717−727.

Kanematsu, S.; Asada, K. Characteristic amino acid sequences of chloroplast and cytosol isozymes of Cu/Zn superoxide dismutase in spinach, rice and horsetail. *Plant Cell Physiol.* **1990**, *31*, 99−112.

Karpinski, S.; Wingsle, G.; Olsson, O.; Haellgren, J. E. Characterization of cDNA encoding Cu,Zn-superoxide dismutases in Scots pine. *Plant Mol. Biol.* **1992**, *18*, 545−555.

Lin, C. T.; Yeh, K. W.; Kao, M. C.; Shaw, J. F. Cloning and characterization of a cDNA encoding the cytosolic copper/zinc-superoxide dismutase from sweet potato tuberous root. *Plant Mol. Biol.* **1993**, *23*, 911−913.

Lin, C. T.; Lin, M. T.; Chen, Y. T.; Shaw, J. F. The gene structure of Cu/Zn-superoxide dismutase from sweet potato. *Plant Physiol.* **1995a**, *108*, 827−828.

Lin, C. T.; Lin, M. T.; Chen, Y. T.; Shaw, J. F. Subunit interaction enhances enzyme activity and stability of sweet potato cytosolic Cu/Zn-superoxide dismutase purified by a His-tagged recombinant protein method. *Plant Mol. Biol.* **1995b**, *28*, 303−311.

Lin, C. T.; Lin, M. T.; Shaw J. F. Cloning and characterization of a cDNA for manganese superoxide dismutase from callus of sweet potato. *J. Agric. Food Chem*. **1997**, *45*, 521−525.

Lin, M. T.; Hwang, Y. S.; Lin, C. T. The significance of Arg-141 to Ser in sweet potato Cu/Zn-superoxide dismutase by site-directed mutagenesis. *J. Chin. Agric. Chem. Soc.* **1996**, *34*, 509−517.

Lütcke, H. A.; Chow, K. C.; Mickel, F. S.; Moss, K. A.; Kern, H. F.; Scheele, G. A. Selection of AUG initiation codons differs in plants and animals. *EMBO J.* **1987**, *6*, 43−48.

McKersie, B. D.; Bowley, S. R.; Harjanto, E.; Leprince, O. Water-deficit tolerance and field performance of transgenic alfalfa overexpression superoxide dismutase. *Plant Physiol.* **1996**, *111*, 1177−1181.

Oseki, H.; Umesono, K.; Inokuchi, H. The chloroplast genome of plants: a unique origin. *Genome* **1989**, *31*, 169−174.

Perl-Treves, R.; Galun, E. The tomato Cu/Zn superoxide dismutase genes are developmentally regulated and respond to light and stress. *Plant Mol. Biol.* **1991**, *17*, 745−760.

Sakamoto, A.; Ohsuga, H.; Tanaka, K. Nucleotide sequences of two cDNA clones encoding different Cu/Zn-superoxide dismutases expressed in developing rice seed. *Plant Mol. Biol.* **1992**, *19*, 323−327.

Sakamoto, A.; Okumura, T.; Kaminaka, H.; Sumi, K.; Tanaka, K. Structure and differential response to abscisic acid of two promoters for the cytosolic copper/zinc-superoxide dismutase genes, *SodCc1* and *SodCc2*, in rice protoplasts. *FEBS Lett.* **1995**, *358*, 62−66.

Sen Gupta, A.; Heinen, J. L.; Holaday, A. S.; Burke, J. J.; Allen, R. D. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1629−1633.

Smith, M. W.; Doolittle, R. F. A comparison of evolutionary rates of the two major kinds of superoxide dismutase. *J Mol. Evol.* **1992**, *34*, 175−184.

Streller, S.; Karpinski, S.; Hällgren, J.-E.; Wingsle, G. Four cytosolic-type CuZn-superoxide dismutases in germinating seeds of *Pinus sylvestris*. *Physiol. Plant* **1994**, *92*, 443−450.

Tanaka, K.; Takio, S.; Yamamota, I.; Satoh, T. Purification of the cytosolic Cu/Zn-superoxide dismutase (Cu/Zn−SOD) of *Marchantia paleacea* var. *diptera* and its resemblance to Cu/Zn−SOD from chloroplasts. *Plant Cell Physiol*. **1996**, *37*, 523−529.

Yeh, K. W.; Juan, R. H.; Su, J. C. A rapid and efficient method for RNA isolation from plants with high carbohydrate content. *Focus* **1991**, *13*, 102−103.

Received for review August 11, 1997. Revised manuscript received October 14, 1997. Accepted October 23, 1997.[⊗] This work was partially supported by the National Science Council of the Republic of China through Grant NSC 87-2311-B-019-001-A18 to C.T.L.

JF9706843

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1997.