Plant Molecular Biology **23**: 911–913, 1993.

Short communication

Cloning and characterization of a cDNA encoding the cytosolic copper/zinc-superoxide dismutase from sweet potato tuberous root

Chi-Tsai Lin[1], Kai-Wun Yeh[2], Ming-Ching Kao[3] and Jei-Fu Shaw[4]
[1] *Department of Bioengineering, Tatung Institute of Technology, 40 Chungshan North Road, 3rd sec. Taipei, Taiwan;* [2] *Department of Botany, National Taiwan University, Taipei, Taiwan;* [3] *Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan;* [4] *Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan*

Received 1 July 1993; accepted in revised form 6 September 1993

Key words: cDNA, cytosolic Cu/Zn-SOD, superoxide dismutase, sweet potato, *Iopomoea batatas* (L.)

Abstract

A full-length cDNA clone encoding a putative copper/zinc-superoxide dismutase (SOD) of sweet potato, *Ipomoea batatas* (L.) Lam. cv Tainong 57, was isolated from a cDNA library constructed in λgt10 from tuber root mRNA. Nucleotide sequence analysis of this cDNA clone revealed that it comprises a complete open reading frame coding for 152 amino acid residues. The deduced amino acid sequence showed higher homology (78–86%) with the sequence of the cytosolic SOD than that of the chloroplast SOD from other plant species. The residues required for coordinating copper and zinc are conserved as they are among all reported Cu/Zn-SOD sequences. In addition, it lacks recognizable plastic or mitochondrial targeting sequences. These data suggest that the isolated sweet potato clone encodes a cytosolic Cu/Zn-SOD.

One of the natural antioxidant enzymes, superoxide dismutase (SOD; superoxide:superoxide oxidoreductase, EC 1.15.1.1), scavenges O_2^-, and thus prevents the lethal effects of O_2^-. SODs are metalloproteins and are classified into three types (Mn-, Fe- and Cu/Zn-SOD), depending on the metal found in the active site. In higher plants, the most prominent SODs are Cu/Zn isozymes found in the cytosol and plastids [8]. It has been observed that the activity of plant SOD increases in response to a variety of environmental and chemical stimuli [3, 7]. Many plant SOD cDNAs from leaf or seedling have been sequenced and compared, but no reports on SOD cDNA from root tissue. We report in this paper the cDNA sequence and deduced amino acid sequence from a cytosolic Cu/Zn-SOD cDNA clone.

Young growing roots of sweet potato, *Ipomoea batatas* (L.) Lam. cv. Tainong 57, were harvested immediately before use. Skinned and diced roots were frozen in liquid nigrogen and ground to powder in a ceramic mortar. Total RNA were pre-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X73139.

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                                                                                GGGGTGCTCTGAGATCACAAACAAAA

 27 ATG GTG AAG GCT GTC GCA GTT CTT AGC AGT AGT GAA GGT GTC AGC GGC ACC ATT TTC TTC
  1  M   V   K   A   V   A   V   L   S   S   S   E   G   V   S   G   T   I   F   F

 87 AGC CAA GAA GGA GAT GGT CCA ACC ACA GTC ACT GGA AAC GTT TCG GGC CTC AAA CCT GGT
 21  S   Q   E   G   D   G   P   T   T   V   T   G   N   V   S   G   L   K   P   G

147 CTT CAT GGC TTC CAT GTC CAT GCC CTA GGT GAC ACA ACA AAT GGA TGC ATG TCT ACT GGA
 41  L   H   G   F   H   V   H   A   L   G   D   T   T   N   G   C   M   S   T   G

207 CCA CAT TTC AAT CCT GCT GGA AAG GAG CAT GGA GCT CCT GGA GAC GAT AAC CGC CAT GCC
 61  P   H   F   N   P   A   G   K   E   H   G   A   P   G   D   D   N   R   H   A

267 GGT GAT CTT GGA AAC ATC ACG GTT GGA GAA GAT GGT ACT GCT TCA TTC ACC ATC ACT GAC
 81  G   D   L   G   N   I   T   V   G   E   D   G   T   A   S   F   T   I   T   D

327 AAG CAG ATT CCG CTT ACT GGA GCA AAT TCT GTT ATT GGA AGA GCT GTT GTT GTT CAT GGT
101  K   Q   I   P   L   T   G   A   N   S   V   I   G   R   A   V   V   V   H   G

387 GAT CCC GAT GAT CTT GGT AAA GGT GGC CAT GAG CTC AGC AAA AGC ACT GGA AAT GCT GGC
121  D   P   D   D   L   G   K   G   G   H   E   L   S   K   S   T   G   N   A   G

447 GGG AGG GTT GCC TGC GGT ATC ATT GGC CTG CAG GGT TAAGGTGTGATTTGCTTATGAAGTCACCCAT
141  G   R   V   A   C   G   I   I   G   L   Q   G   *

514 GGCAGAGAGGAAAAAGCTTGAATAAAGGAATCATTTTGTGCAGTGCTTTTTCTGTGTTTTTGAACTTCATGCCTTGACC
593 AACTCTTATGGGGGATAGAAACTTTGATATGTATTCACTGTTTATCGTACTTCATTTTGCCTAATTACTATCTGAATAA
672 TAAGTTGGTTTGTGAGAAAAAAAAAAAAAAA
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Fig. 1. Nucleotide sequence of sweet potato SOD (SW-SOD) cDNA and the deduced amino acid sequence. Numbers to the left refer to nucleotide and amino acid residuces. Consensus sequences of the translation start site and polyadenylation signal are underlined. The asterisk denotes the stop signal.

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                                                                  * *
SW-SOD    MVKAVAVLSS SEGVSGTIFF SQEGDGPTTV TGNVSGLKPG LHGFHVHALG  50
TSOD      ........N. .......YL. T.V.VA.... N..I...... ..........
MSOD-4    ........G. ....K..... T......... ..S...... ..........
MSOD-2    ........AG TD-.K..... .......... ..SI...... ..........
RSODA     .....V..G. ..I.K...H. V......... ..S...... .....I....
                     *       *          *   *
SW-SOD    DTTNGCMSTG PHFNPAGKEH GAPGDDNRHA GDLGNITVGE DGTASFTITD 100
TSOD      .......... ..Y....... ...E.EV... .......... ..........
MSOD-4    .......... ..Y...S... ...E.E.... .....V.A.A ..V.NINV..
MSOD-2    .......... .....V.L.. ...EFED... .....V.A.. ..VVNVN...
RSODA     .......... ..Y....... ...E.ET... .....V.A.. ..V.NIHVV.
                               *
SW-SOD    KQIPLTGANS VIGRAVVVHG DPDDLGKGGH ELSKSTGNAG GRVACGIIGL 150
TSOD      .......PQ. I.........A .......... .......... ..I.......
MSOD-4    S......P.. I.........A .......... .......... ..........
MSOD-2    S....A.PH. I.........A .......... .......... ..........
RSODA     S......P.. I.........A .......... ....T..... ..........


SW-SOD    QG 152
TSOD      ..
MSOD-4    ..
MSOD-2    ..
RSODA     ..
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Fig. 2. Comparison of amino acid sequences of SODs: SW-SOD, sweet potato SOD (this study); TSOD, tomato SOD [6]; MSOD-4, maize SOD-4 [2]; MSOD-2, maize SOD-2 [1]; RSODA, rice SODA [8]. Numbers refer to SW-SOD. Three sequences are shown only where they differ from SW-SOD. A dot refers to identities with SW-SOD. A dash denotes deletion. Residues coordinating copper and zinc are indicated with asterisks. The two cysteines that form a disulfide bridge are boxed.

pared by the guanidium HCl procedure [4]. The poly(A)$^+$ RNAs were isolated according to the oligo-(dT) affinity method [4] and then ligated with λgt10. The recombinant cDNAs were packaged with Stratagene's Gigapack and then a cDNA library was constructed by using C600hlf as the host. Putative positive clones were selected by plaque hybridization with ^{32}P-labelled DNA fragment of *Aspergillus japonicus* SOD (unpublished data) which was amplified by PCR using two primers (5′-TCCATGGGTTCCATGTGC-3′, 5′-GTTTCCGGTGCTCTTGCT-3′) according to the sequence of maize SOD-4 [2]. A cDNA fragment from purified positive plaques was subcloned into pGEM-7zf(+) (Promega) named SW-SOD-15 using *Escherichia coli* JM109 as host. Nucleotide sequence was determined in both directions by the automated fluorescent sequencing of DNA with dye primers using the Applied Biosystems Model 373A DNA sequencing system (Applied Biosystems).

Figure 1 shows the nucleotide and deduced amino acid sequences of one cDNA clone. Sequencing analysis found that the cDNA was full-length, comprising a complete open reading frame coding for 152 amino acid residues and a polyadenylated residuce on 3′ end. There is no transit peptide as reported in chloroplast or mitochondria enzymes. The DNA sequence translation start site (AAAAATGG) matches the consensus sequence (AACAATGG) reported for this region in plants [5].

The deduced sequence of 152 amino acids showed higher homology with the sequences of the cytosolic SOD from several other plant species (maize SOD-2, 78.3% [1]; maize SOD-4, 83.6% [2]; rice RSODA, 79.0% [8], rice RSODB, 83% [8]; tomato TSOD, 85.5% [6]) than the sequence of chloroplastic counterpart (pea SOD, 60.5% [10]; petunia SOD, 62.5% [11]; tomato SOD, 63.2% [6]). This suggests that the sweet potato SOD is of a cytosolic type. Figure 2 shows that seven residues coordinating copper (histidine 45, 47, 62 and 119) and zinc (histidine 62, 70, 79 and aspartate 82), as well as the two cysteines (56 and 145) that form a single disulfide bridge, are conserved as they are among all reported Cu/Zn-SOD sequences [3].

Acknowledgements

This work was supported by the National Science Council of the Republic of China. We thank Mr. Ming-Tse Lin for his excellent technical assistance.

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