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Plant Gene Register

Cloning and Sequencing Analysis of a Complementary DNA for Manganese-Superoxide Dismutase from Rice (*Oryza sativa* L.)[1]

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For aerobic life oxidative stress is an inevitable misfortune that is mediated by the high reactivity of active oxygen species. One of the detrimental species is the superoxide anion radical O_2^-. In biological systems this radical is disproportionated into molecular oxygen and hydrogen peroxide by the catalytic action of the enzyme SOD (superoxide:superoxide oxidoreductase; EC 1.15.1.1). SOD is a group of metalloproteins holding either manganese (Mn-SOD), iron (Fe-SOD), or copper/zinc (Cu/Zn-SOD) as the prosthetic metal. Cu/Zn- and Mn-SODs are widely distributed in the plant world, whereas Fe-SOD has so far been identified only in a few species (Asada et al., 1980; Van Camp et al., 1990). Cu/Zn-SOD, the major isoform in plant cells, has cytosolic and plastidic locations. Mn-SOD exists as a minor class principally destined for the mitochondrial matrix. However, it has been reported that Mn-SOD is associated with the thylakoid membrane in spinach chloroplasts (Hayakawa et al., 1985) and the peroxisome-localized Mn-SOD has also been found in pea leaves (Sandalio et al., 1987).

In previous studies carried out in our laboratory, two cDNA sequences and corresponding nuclear genes for Cu/Zn-SODs were characterized from rice (*Oryza sativa* L.) plants (Sakamoto et al., 1992a, 1992b). The current communication describes the isolation of a cDNA (Table I) encoding the putative precursor to mitochondrial Mn-SOD from rice seeds by a combination of PCR and library screening techniques.

A λgt10 cDNA library of green leaves of maize (a kind gift from Dr. K. Izui, Kyoto University) was amplified by PCR using oligonucleotide primers designed for the maize Mn-SOD cDNA (White and Scandalios, 1988). The PCR product with the expected size was sequenced to confirm its identity and used as the probe to screen a cDNA library in λgt11 made from poly(A)$^+$ RNA from immature rice seeds. The insert from the longest cDNA clone, M14, was subcloned into pBluescript SK$^+$ vector and its nucleotide sequence was determined on both strands. The cDNA is 878 bp in length except for the 3′-terminal polyadenylated residues and contains an open reading frame encoding a polypeptide of 231 amino acids. The NH_2-terminal 19 residues of rice Mn-SOD protein (subcellular location unknown) has been deposited in the PIR protein data base (the accession number is PS0186; Kamo and Tsugita). The sequence encoded by the cDNA was found to comprise the corresponding residues at positions 28 to 46, although one mismatch occurred at position 29 of the deduced amino acid sequence. No sequence ambiguity was observed in the region between the M14 cDNA and the corresponding gene (A. Sakamoto, Y. Nosaka, and K. Tanaka, unpublished results), indicating that this gene encodes Thr rather than Glu at the 29th codon.

It should be noted that Kanematsu and Asada (1989) detected two distinct Mn-SOD activities in rice seeds, which may account for the amino acid discrepancy between the predicted sequence from the cDNA and the NH_2-terminal sequence of the protein. The sequence alignment assigned the putative signal sequence (positions 1–27) of the rice Mn-SOD precursor for organelle targeting. This 27-residue polypeptide is composed mostly of hydrophobic amino acids (especially Ala and Leu) with four positive-charged residues (Arg and Lys), but it was devoid of acidic residues. We assumed the polypeptide to be the mitochondrial presequence because it shared significant sequence identity to that of the maize mitochondrial Mn-SOD (White and Scandalios, 1988). The rice Mn-SOD precursor also showed a high degree of

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Abbreviations: PCR, polymerase chain reaction; SOD, superoxide dismutase.

Table I. *Characteristics of a cDNA for Mn-SOD from rice*

Organism:
Oryza sativa L. (rice) cv Nipponbare.
Location in Genome:
Nuclear genome; chromosome location unknown.
Gene, Product, Function:
Rice Mn-SOD gene; precursor to Mn-SOD (EC 1.15.1.1); dismutation of superoxide anion radicals into hydrogen peroxide and molecular oxygen.
Clone Designation:
M14.
Source:
λgt11 cDNA library constructed from poly(A)$^+$ RNA of immature rice seed.
Techniques:
PCR; cDNA cloning in λgt11 system; restriction subcloning into pBluescript SK$^+$ (Stratagene); dideoxy sequencing of both strands.
Method of Isolation, Subsequent Identification:
Hybridization with the amplified maize Mn-SOD cDNA fragment; agreement of the encoded sequence by the cDNA with the NH_2-terminal sequence of rice Mn-SOD protein (PIR data base, PS0186); sequence identity with deduced amino acid sequences of Mn-SODs from maize (White and Scandalios, 1988), tobacco (Bowler et al., 1988), and pea (Wong-Vega et al., 1991) (90.2, 77.9, and 74.5% identity, respectively, within the mature polypeptide region).
Expression Characteristics:
Transcript (about 1000 nucleotides) as detected on northern blots of leaves, roots, seeds, and calli.
Feature of the cDNA Structure:
Open reading frame of 693 bp flanked 5′ by 28 bp and 3′ by 157 bp of untranslated sequences, respectively; translational start site at nucleotide 29 and termination site at nucleotide 722.
Codon Usage:
Third base frequency: C > G > T > A.
(G + C) Content:
55.2% overall; 58.2% in the protein-coding region.
Structural Features of the Protein:
Open reading frame encodes 231 amino acids with a predicted M_r of 24,997; first 27 residues correspond to a putative mitochondrial presequence.
Antibodies:
Not available in our laboratory.
Subcellular location:
Not determined, but possibly mitochondrial matrix (based on the presence of a putative signal sequence and its homology to the maize mitochondrial Mn-SOD presequence).

sequence identity with those reported for maize, tobacco (Bowler et al., 1988), and pea (Wong-Vega et al., 1991) within the mature polypeptide region (90.2, 77.9, and 74.5%, respectively).

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