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Plant Gene Register

The Gene Structure of Cu/Zn-Superoxide Dismutase from Sweet Potato[1]

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SODs (superoxide:superoxide oxidoreductase, EC 1.15.1.1) catalyze the dismutation of superoxide to dioxygen and hydrogen peroxide to protect organisms from oxidative damage (Hassan, 1984). SODs are metalloproteins that are classified into three types (Mn-, Fe-, and Cu/Zn-SOD) depending on the metal found in the active site. In plants, the most prominent SODs are Cu/Zn isozymes. It has been shown that transgenic plants that overexpress chloroplastic Cu/Zn-SOD increase resistance to oxidative stress (Gupta et al., 1993) and the activity of plant SOD increases in response to a variety of environmental and chemical stimuli (Fridovich, 1986; Perl-Treves and Galun, 1991). Recently, increased levels of SOD activities resulting from differential regulation of individual SOD genes at the transcriptional level were also reported (Perl-Treves and Galun, 1991). Many plant SOD cDNAs from leaf or seedling have been studied, but information concerning the Cu/Zn-SOD gene from root tissue is limited. Previously, we cloned and sequenced a full-length cytosolic Cu/Zn-SOD cDNA from sweet potato root (Lin et al., 1993). In this paper, we report the structural features of a Cu/Zn-SOD gene from the same tissue (Table I).

Positive clones derived from the cytosolic Cu/Zn-SOD gene were characterized. The total sequence is 3950 bp long. Structural alignment showed perfect agreement between the cDNA sequence and the open reading frame of genomic DNA constructed from eight exons. The coding sequence, comprising 456 bp, begins in the second and ends in the last exon.

The genomic sequence was compared with SOD gene structures derived from eukayotes: human (2.3 kb, five exons, four introns, Levanon et al., 1985), *Drosophila melanogaster* (1.8 kb, two exons, one intron, Seto et al., 1987), *Neurospora crassa* (1.0 kb, four exons, three introns, Chary et al., 1990), and rice (2.0 kb, eight exons, seven introns, Sakamoto et al., 1992). It has been observed that the exon/intron organization of Cu/Zn-SOD from sweet potato (*Ipomoea batatas L.*) is similar to rice's genomic sequence but differs from those found from other sources, especially in terms of numbers of introns and splicing sites. Although exon/intron organization of Cu/Zn-SOD is similar between sweet potato and rice, the lengths of introns in sweet potato are much greater than those in rice.

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Abbreviation: SOD, superoxide dismutase.

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Table I. *Characteristics of the gene structure of Cu/Zn-SOD from sweet potato*

Organism:
Ipomoea batatas (L.) Lam cv Tainong 57.
Chromosomal Location:
Not determined.
Gene Copy Number
Not determined.
Gene Product:
Cytosolic Cu/Zn-SOD.
Function:
Catalyze the dismutation of superoxide to dioxygen and hydrogen peroxide.
Source:
Genomic libraries were constructed in λgt11 using chromosomal DNA from sweet potato tuberous root.
Isolation:
Positive clones were screened by plaque hybridization with the ^{32}P-labeled probes derived from SW-SOD cDNA fragment (Lin et al., 1993). Restriction mapping revealed that one positive clone (λsod8–2) covering the whole cytosolic Cu/Zn-SOD gene was cut with *Eco*RI, and the insert DNA (6 kb) was recovered with an NA-45 membrane from 0.6% agarose. The insert DNA was cut with *Sac*I to 3.2-, 1.8-, and 1.0-kb fragments. Only the 1.8-kb DNA fragment was successfully subcloned to pGEM-7zf(+); it was sequenced and found to be the fragment covering the 5′-end of the gene. So, we synthesized appropriate primers according to the cDNA sequence and used the 6.0-kb insert fragment as a template to amplify the other parts of the gene by the PCR technique. Two DNA fragments (0.6 and 1.6 kb) were amplified and subcloned to pGEM-7zf(+). Sequence analysis indicated that the two fragments encompass the other parts of the gene.
Method of Identification:
Sequence comparison with its cDNA and the rice SOD gene (Sakamoto et al., 1992).
Sequencing Methods:
Restriction fragments and PCR products were subcloned in pGEM-7Zf(+). Appropriate primers were synthesized to sequence both strands by the dideoxy technique using Sequenase (United States Biochemical).
Determination of the Transcription Initiation Site:
A 25-mer oligonucleotide complementary to a 5′ region of the SOD gene was synthesized and labeled with ^{32}P using T4 polynucleotide kinase. The radioactive oligonucleotide was annealed to poly(A)$^+$ RNA (Lin et al., 1993) and extension was conducted using a kit (Promega). The product was run on a polyacrylamide sequencing gel alongside a complete dideoxy sequencing reaction product primed on a genomic clone with the same oligonucleotide.
Feature of Gene Structure:
The cytosolic Cu/Zn-SOD gene is split into eight exons and seven introns spread in 3950 bp of chromosomal DNA, with the coding sequence beginning in the second and ending in the eighth exon. The CAAT and TATA boxes are at −88 and −64 bases upstream from the transcriptional start site, respectively, and there are two GC-rich regions at about 60 and 80 bp upstream from the CAAT box.