cDNA and Derived Amino Acid Sequence of the Chloroplastic Copper/Zinc-Superoxide Dismutase from Aspen (Populus tremuloides)\(^1\)

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All aerobic organisms and many anaerobic organisms are subject to oxidative stress resulting from the deleterious effects of reduced oxygen species. SOD has been identified as a key enzyme in protecting the cells from oxidative damage by catalyzing the dismutation of superoxide radicals (\(O_2^-\)) to hydrogen peroxide and molecular oxygen (Fridovich, 1986). In the chloroplasts, superoxide radicals are produced during the univalent reduction of dioxygen during photosynthetic electron transport (Asada et al., 1974).

Three types of SODs have been classified according to the metal present at the catalytic site: Cu/Zn-SOD, Mn-SOD, and Fe-SOD. Plants generally contain Cu/Zn-SOD in the cytosol, Fe-SOD and Cu/Zn-SOD in the chloroplast, and Mn-SOD in the mitochondrial matrix (Bannister et al., 1987). The Cu/Zn-SOD is the most prevalent SOD in plants and most of it is localized in the chloroplast in green leaves.

Ozone is an industrial pollutant and is a known stress factor that brings about the production of reactive oxygen species that can cause extensive damage within the cell (Gupta et al., 1991). We have been working with three clones of aspen that respond differently to ozone stress ranging from highly tolerant to highly sensitive (Kamosky et al., 1992). Our preliminary results suggest that ozone-tolerant clones display higher amounts of SOD activity than the sensitive clones. We are interested in studying ozone-related expression of SOD in aspen, and to this end we have cloned and sequenced the chloroplastic SOD from aspen, a hardwood tree species (Table I).

A cDNA library was derived from poly(A\(^+\)) mRNA of ozone-fumigated young aspen leaves of ozone-tolerant clones. cDNA synthesis was carried out using the Superscript cDNA synthesis system (GIBCO BRL). The library was cloned into a λgt22 system and screened using a 875-bp pea chloroplastic Cu/Zn-SOD cDNA clone, pSpA2-4 (Scioli and Zilinskas, 1988). A cDNA clone containing an 800-bp insert, which hybridized to the radiolabeled pea SOD, was isolated and subcloned into a pGEM 5Z vector to generate a nested set of deletions using the Erase-a-Base system (Promega). The cDNA clone was sequenced by the Sanger’s dideoxy method (Sanger et al., 1977) using a Sequenase kit (United States Biochemical).

The full-length clone sequenced contains 730 bp, flanked 5' by 54 bases of noncoding region and 3' by 68 bases. The open reading frame contains coding information for the mature subunit of a chloroplastic Cu/Zn-SOD, which encodes a 21.5-kD protein. In addition, the cDNA encodes a transit peptide of 48 amino acid residues at the amino terminus. The transit peptide sequence contains high amounts of basic hydrophobic, hydroxylated amino acids lacking negatively charged residues, which are characteristic of all known transit peptides (Schmidt and Mishkind, 1986). We found the Cu/Zn-metal binding signatures at amino acid positions 109, 129, and 166 in the sequence.

Abbreviation: SOD, superoxide dismutase.

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Table I. Characteristics of the chloroplastic Cu/Zn-SOD from aspen

| Organism: | Populus tremuloides L. Michx. (quaking aspen). |
| Gene Product: | Chloroplastic Cu/Zn-SOD (EC 1.15.1.1). |
| Clone Type: | cDNA, full length. |
| Source: | cDNA library in λgt22 constructed from ozone-fumigated leaves of ozone-tolerant aspen clone No. 216. |
| Techniques: | cDNA library was screened using radiolabeled pea chloroplastic Cu/Zn-SOD; dideoxy sequencing of both strands using United States Biochemical Sequenase kit. |
| Method of Identification: | Sequence homology with other SOD sequences. The aspen clone has 95% homology with pea chloroplastic Cu/Zn-SOD and a close homology with those from spinach, pine, and Arabidopsis. |
| Features of CDNA Clone: | Contains an open reading frame of 201 amino acids; molecular mass of 21.5 kD; 48-amino acid transit peptide sequence amino-terminal to the mature protein sequence. No poly(A) tail seen. |
| Subcellular Location: | Chloroplast. |

\(^{1}\)This work was funded by the Michigan Research Excellence Fund grant to D.F.K. and G.K.P. 

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The coding sequence of chloroplastic Cu/Zn-SOD seems to be highly conserved among plant species. We observed 95% sequence similarity with the pea chloroplastic Cu/Zn-SOD, which was used as a probe to isolate our cDNA clone. Sequence comparisons with other species from the database (Bilofsky and Burks, 1988) showed 72% homology with spinach Cu/Zn-SOD, 55% with the cytosolic isozyme from spinach, 58% with pine Cu/Zn-chloroplastic SOD, 53% with the cytosolic isozyme from pine, 56% with tobacco cytosolic Cu/Zn-SOD, 55% with Arabidopsis cytosolic Cu/Zn-SOD, and 56% with pea cytosolic Cu/Zn-SOD.

ACKNOWLEDGMENT

The authors would like to thank Dr. B. Zilinskas for kindly providing the pea SOD (PSPA2-4) clone.

Received May 13, 1994; accepted June 13, 1994.

LITERATURE CITED


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