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*Plant Gene Register*

# Molecular Cloning and Nucleotide Sequence of a Lipoxygenase cDNA from Ripening Tomato Fruit[1]

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LOXs (EC 1.13.11.12) are nonheme iron-containing dioxygenases that catalyze the incorporation of molecular oxygen into unsaturated fatty acids with a *cis,cis*-1,4-pentadiene structure. In higher plants, the resultant hydroperoxide product serves as an intermediate in the production of volatile aromatic compounds such as hexenal, jasmonic acid, and traumatin (Vick and Zimmerman, 1987). Mutiple isoforms of LOX have been observed in most plant tissues, including tomato (*Lycopersicon esculentum*) fruit (Siedow, 1991; Bowsher et al., 1992; Droillard et al., 1993). The function of various LOX isoforms in plants is unknown, but their roles during all stages of plant growth and development have been suggested (Hildebrand, 1989; Siedow, 1991). LOX multigene families have been characterized in several plant species, including soybean, pea, and rice (Shibata et al., 1988; Ealing and Casey, 1989; Steczko et al., 1992; Peng et al., 1994).

A cDNA library, constructed from poly($A^+$) RNA isolated from red-ripe tomato fruit, was immunoscreened with polyclonal antibodies raised against an 89-kD protein that accumulated maximally during fruit ripening (Kausch and Handa, 1993). Among the 24 cDNA clones obtained, 17 were nearly identical, based on DNA sequence analysis and shared significant homology to the LOX gene family (EMBL and GenBank data bases). The full-length cDNA clone was isolated by a subsequent screening of the cDNA library using the insert from the largest cDNA clone as a probe. The sequenced cDNA clone contains 2871 bp and an open reading frame encoding a protein of 859 amino acids with a calculated molecular mass of 97 kD and a pI of 5.5 (Table I). Sequence data base searches using the BLAST algorithm program (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD) revealed 50 to 70% identity at the nucleic acid level and between 60 and 80% similarity at the amino acid level to previously reported LOX genes from plants. The deduced amino acid sequence contains the conserved 39-amino acid motif present in all reported LOXs (Steczko et al., 1992).

**Table I.** *Characteristics of a tomato cDNA encoding LOX*

Organism:
*Lycopersicon esculentum* Mill. cv Rutgers.
Function:
Hydroperoxidation of unsaturated fatty acids having a *cis,cis*-1,4-pentadiene structure with oxygen.
Source:
cDNA library constructed in λ-ZAP (Stratagene) by reverse transcription from poly($A^+$) RNA isolated from the red-ripe stage of tomato fruit ripening.
Method of Cloning:
Polyclonal antibodies raised against the purified 89-kD protein were used to immunoscreen the cDNA library.
Method of Sequencing:
Nested deletions of the largest clone obtained (2871 bp) were made by *Exo*III nuclease treatment (Promega's Erase-a-Base System) for both strands and sequenced with Sanger's dideoxynucleotide chain termination method using Sequenase version 2.0 (Amersham Life Science).
Features of the cDNA Clone:
The entire length of the cDNA contains 2871 nucleotides with an open reading frame of 2577 nucleotides and has 44 and 250 nucleotides of 5′ and 3′ untranslated regions, respectively.
Features of the Encoded Protein:
The calculated molecular mass is 97 kD and the pI is 5.5.

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Abbreviation: LOX, lipoxygenase.

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