LOX (EC 1.13.11.12) are ubiquitous plant enzymes that catalyze the hydroperoxidation of polyunsaturated fatty acids; potato tubers contain high levels of LOX (Pinsky et al., 1971). LOX are believed to catalyze a key step in the formation of jasmonates, which have profound effects on plant physiology and gene expression (Staswick, 1992), including the induction of tuber formation in potatoes (Koda et al., 1991). LOX have also been implicated in the arachidonic acid-elicited hypersensitive cell death response of potato discs and callus lines (Bostock et al., 1992; Vaughn and Lulai, 1992).

The potato tuber LOX that has been purified effects hydroperoxidation predominantly at the 5-carbon of arachidonic acid (Shimizu et al., 1990) and thus differs in positional specificity from the well-characterized soybean seed arachidonate 15-LOX (Boyington et al., 1993); analysis of its structure may afford clues to the basis of positional specificity of hydroperoxidation by plant LOX.

This report describes lox1:St:1, a Solanum tuberosum LOX cDNA that corresponds to mRNA that is abundant in developing tubers. The sequence, described in Table I, includes 200 bp between the stop codon and the poly(A) tail, and 1 bp 5' to the initiation codon. The proposed initiating ATG aligns well with other, homologous plant LOX sequences; primer extension using an oligonucleotide corresponding to nucleotides 2 to 22 indicates that the potato tuber LOX mRNA has 74 nucleotides of 5' noncoding sequence. The amino acid sequence predicted by lox1:St:1 is more similar to the Arabidopsis thaliana LOX 1 sequence reported by Melan et al. (1994) than to any other published LOX sequence, showing 80% similarity (66% identity) to Arabidopsis LOX 1. Boyington et al. (1993) described an internal cavity (II) in soybean 15-LOX that is likely to accommodate the fatty acid substrate. Within this cavity they described 14 highly conserved amino acids; these are also conserved in the sequence predicted from lox1:St:1. One residue (Phe^357) in the cavity may be involved in the determination of positional specificity (see Boyington et al., 1993). This residue is Phe in all plant LOXs so far reported with three exceptions: that predicted from lox1:St:1, LOX 1 from Arabidopsis (Melan et al., 1994), and LOX L-2 from rice (Ohta et al., 1992), where it is Val in all cases. Structure determination of the purified LOX will determine how significant this is to positional specificity.

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LITERATURE CITED


Table 1. Characteristics of the potato tuber LOX cDNA

Organism:
Potato (Solanum tuberosum L. cv Desiree).

Function:
LOX; fatty acid hydroperoxidation.

Techniques:

lox1:St:1 was obtained by screening a λgt11 cDNA library, representing mRNA from developing tubers, with a subfragment of a part-length cDNA clone, as follows. The part-length cDNA clone (AE-14) was isolated from a potato tuber λgt10 cDNA library by plaque hybridization with the central EcoRl fragment from the insert of the pea seed LOX cDNA clone pPE923 (see Ealing and Casey, 1989) at low stringency (hybridization 3X SSC, 4X Denhardt's solution, 65°C; washes 2X SSC, 50°C). The 5' BamHI-Pstl fragment (200 bp) from AE-14 was used to select CC-16 from the λgt11 cDNA library by hybridization at high stringency (washes at 0.1X SSC, 65°C). The sequence of lox:St:1 (the insert in CG-16 [2809 bp]) was determined by primer walking/dideoxy sequencing of EcoRI fragments cloned into Bluescript KS+, using an automated (Pharmacia) DNA sequencer, and by manual cycle sequencing of phage DNA, using 32P-labeled oligonucleotides and Taq polymerase. Both strands were sequenced at least twice.

Sequence Identification:
Comparison with several plant LOX cDNA and gene sequences.

Expression and Regulation:
The mRNA corresponding to lox1:St:1 is produced throughout tuber development and is relatively highly abundant (approximately 0.5 ng mRNA/μg total RNA, measured using in vitro transcripts as standards). Several slightly different sequences were identified in each library by DNA sequencing, suggesting the existence of several expressed genes for potato tuber LOX.

Features of the Predicted Amino Acid Sequence:
Eighty percent similar to the sequence predicted by the LOX1 gene from A. thaliana (Melan et al., 1994); each has an insertion of five amino acids at position 249 (in the potato sequence) relative to other predicted plant LOX sequences. The amino acids that are liganded to Fe in soybean seed arachidonate 15-LOX (Boyington et al., 1993) are conserved in the potato tuber LOX.