Plant Physiol. (1995) 107: 265–266

Plant Gene Register

Sequence of a cDNA Clone Encoding a Potato (*Solanum tuberosum*) Tuber Lipoxygenase[1]

Rod Casey*

Department of Applied Genetics, John Innes Centre, Colney Lane, Norwich NR4 7UH, United Kingdom

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^{557}) 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.

[1] This work was supported by a grant-in-aid to the John Innes Institute from the Biotechnological and Biological Sciences Research Council.

* E-mail kc@bbsrc.uk.ac; fax 44–603–56844.

Abbreviation: LOX, lipoxygenase(s).

ACKNOWLEDGMENTS

I thank Anne Edwards and Claire Grierson for access to their cDNA libraries, Jacqui Marshall for plant material, and Colette Forster for help and advice on the use of the automated DNA sequencer.

Received June 6, 1994; accepted June 30, 1994.
Copyright Clearance Center: 0032/0889/95/107/0265/02.
The EMBL/GenBank accession number for the sequence reported in this article is X79107.

LITERATURE CITED

Bostock RM, Yamamoto H, Choi D, Ricker KE, Ward BL (1992). Rapid stimulation of 5-lipoxygenase activity in potato by the fungal elicitor arachidonic acid. Plant Physiol **100:** 1448–1456

Boyington JC, Gaffney BJ, Amzel M (1993) The three-dimensional structure of an arachidonic acid 15-lipoxygenase. Science **260:** 1482–1486

Ealing P, Casey R (1989) The cDNA cloning of a pea (*Pisum sativum*) seed lipoxygenase. Sequence comparisons of the two major pea seed lipoxygenase isoforms. Biochem J **264:** 929–932

Koda Y, Kikuta Y, Tazaki H, Tsujino Y, Sakamura S, Yoshihara T (1991) Potato tuber-inducing activities of jasmonic acid and related compounds. Phytochemistry **30:** 1435–1438

Melan MA, Nemhauser JL, Peterman TK (1994) Structure and sequence of the *Arabidopsis thaliana* lipoxygenase 1 gene. Biochim Biophys Acta **1210:** 377–380

Ohta H, Shirano Y, Tanaka K, Morita Y, Shibata D (1992) cDNA cloning of rice lipoxygenase L-2 and characterization using an active enzyme expressed from the cDNA in *Escherichia coli*. Eur J Biochem **206:** 331–336

Pinsky A, Grossman S, Trop M (1971) Lipoxygenase content and antioxidant activity of some fruits and vegetables. J Food Sci **36:** 571–572

Shimizu T, Honda Z-I, Miki I, Seyama Y, Izumi T, Rådmark O, Samuelsson B (1990) Potato arachidonate 5-lipoxygenase: purification, characterization, and preparation of 5(S)-hydroperoxyeicosatetraenoic acid. Methods Enzymol **187:** 296–306

Staswick PE (1992) Jasmonate, genes, and fragrant signals. Plant Physiol **99:** 804–807

Vaughn SF, Lulai EC (1992) Further evidence that lipoxygenase activity is required for arachidonic acid-elicited hypersensitivity in potato callus cultures. Plant Sci **84:** 91–98

Table I. *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 *Eco*RI fragment from the insert of the pea seed LOX cDNA clone pPE923 (see Ealing and Casey, 1989) at low stringency (hybridization 3× SSC, 4× Denhardt's solution, 65°C; washes 2× SSC, 50°C). The 5′ *Bam*HI-*Pst*I fragment (200 bp) from AE-14 was used to select CG-16 from the λgt11 cDNA library by hybridization at high stringency (washes at 0.1× SSC, 65°C). The sequence of *lox:St:1* (the insert in CG-16 [2809 bp]) was determined by primer walking/dideoxy sequencing of *Eco*RI fragments cloned into Bluescript KS^+, using an automated (Pharmacia) DNA sequencer, and by manual cycle sequencing of phage DNA, using ^{33}P-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.