



Rapid Report

The primary structure of a lipoxygenase from the shoots of etiolated lentil seedlings derived from its cDNA

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Abstract

Screening of a cDNA library constructed from the shoots of etiolated lentil seedlings resulted in finding a 2778 bp cDNA sequence, containing an open reading frame coding for a lipoxygenase of 866 amino acid residues. This lipoxygenase appears to be a novel type of vegetative lipoxygenase, different from the seed lipoxygenases of other leguminosae (complete homology $\leq 72\%$).

Key words: Plant lipoxygenase; cDNA-cloning; Sequence homology; (*Lens culinaris*)

Lipoxygenases (EC 1.13.11.12, linoleate:oxygen oxidoreductase, LOX) contain one non-heme iron and catalyze the dioxygenation of polyunsaturated fatty acids having a 1Z,4Z-pentadiene system. The enzyme is found in a large number of higher plants (for a recent review on plant LOXs see Ref. 1), but also in algae [2], yeast [3] and cyanobacteria [4]. Particularly rich sources of LOX are the seeds of legumes and most work has been directed to these LOXs, including the elucidation of the primary structure of 5 of these seed LOXs [5]. In plants different LOXs are present in different tissues and at different stages of development [6]. High LOX levels are often associated with germination [7] and the response to wounding and pest attacks [8], suggesting that LOX also has a part in the

defence mechanism of plants. Sequence information on these vegetative LOXs is sparse and comprise a full sequence of a LOX from arabidopsis [9], a fragment of PLP1 from pea seedlings [8], fragments of SLA, SLB1 and SLB2 from soybean seedling hypocotyls [8] of which SLA is identical to SLX found in soybean seedling cotyledons [10]. Finally there is an almost complete sequence of BL3 from leaves of Red Mexican bean seedlings [11]. Other available sequences are RL2 from complete rice seedlings [12] and BLA from Red Mexican bean [13].

The LOX activity in lentil (*Lens culinaris*) does not deviate from the pattern generally found in legumes.

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Protein:      525-Ser His Trp Leu Asn Thr His-531
SL1, SL2, SLX: AGC CAT TGG TTA AAT ACT CA
SL3:          --- --- --- --- --C --- --
PL2, PL3:    --- --- --- --G --- --- --
LLOX:        --- --- --- --G --C --- --
  
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Fig. 1. Consensus part of plant lipoxygenase sequences used to design oligonucleotide probes. Upper line: The conserved protein sequence. The numbering refers to the found lentil lipoxygenase sequence. Other lines: corresponding DNA sequences. Nucleotides identical to those in the sequence of SL1 are indicated by dashes (-). In a Northern blot of lentil seedling mRNA the 2 probes complementary to SL3, PL2 and PL3 proved to hybridize at higher stringency than the one corresponding to SL1, SL2 and SLX and were consequently used for screening.

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The nucleotide sequence data reported in this paper will appear in the EMBL, Genbank and DDBJ nucleotide sequence databases under the accession number X71344

Abbreviations: LOX, lipoxygenase; AL, Arabidopsis lipoxygenase; BLA, BL3: Mexican bean lipoxygenases A and 3; LLOX, lentil seedling lipoxygenase; PL2, PL3, PLP1: pea lipoxygenases 2, 3 and P1; RL2, rice lipoxygenase 2; SL1, SL2, SL3, SLA, SLB1, SLB2: soybean lipoxygenases 1, 2, 3, A, B1, B2; SLX, soybean seedling cotyledon lipoxygenase.

gatgcttcattattggttgtagagccaaaactcaagggaaactgtgattttgatgcaaaagaatgtttggacatcaatgcctcac 90
M A S L L F G R G Q K L K G T V I L M Q K N V L D I N A L T 30
 tgcagctcaaagccctagtggtatcatcggtggtgcttttggtgtagcttggtagtattgctggctctataaattgacaccgccaccgcttt 180
A A Q S P S G I I G G A F G V V G S I A G S I I D T A T A F 60
 tttgggtcgctctgctccgtcttaggtgatcagtgctaccggtgctgatgcaagtggaaagggaaaagtgtcaaaagggcattcttggga 270
L G R S V R L R L I S A T V A D A S G K G K V S K E A F L E 90
 aggtcttcttacctccataccaacattgggagacaaaactctgctttcagtggtcattttgaaatgggacagtaacatgggaactccagg 360
G L L T S I P T L G D K Q S A F S V H F E W D S N M G T P G 120
 agctttttatattgagaatttcatgcaaggtggtgaattcttctctgtcagtttgacacttgatgacgttccaaacggttgggaagcatcaa 450
A F Y I E N F M Q G G E F F L V S L T L D D V P N V G S I K 150
 gtttgcttgcaactcatggatttacaatgataaaaagtaccaatccgaccgcatcttcttcccaacaagacatatcttccaagtgccac 540
F A C N S W I Y N D K K Y Q S D R I F F A N K T Y L P S A T 180
 cccggctccattagtgctctatagacaagaagattgaagactctaagaggagacggaactggagagcgtcaagaatgggatagaatata 630
P A P L V S Y R Q E E L K T L R G D G T G E R Q E W D R I Y 210
 tgattacgatgtttacaatgacttgggagctcccgaccaaaagctaccttaggtcgtccagttcttggaggatcaagcaccttgcctta 720
D Y D V Y N D L G A P D Q K A T L G R P V L G G S S T L P X 240
 tcctcgaaggggaagaaccggccgaagaagactgtaaaagagcctcagagtgagagtgcgaagtgcactgtgtatcttccaagagacga 810
P R R G R T G R K K T V K E P Q S E S R S D T V Y L R R D E 270
 agcatttggctcatgtgaagtgcgtccgactttctgtttacatactcaaactcagcatctcaaatatcgtacctcaattgagatctgtagt 900
A F G H V K S S D F L V Y I L K S A S Q N I V P Q L R S V V 300
 tacattacaactcaataccccgagtttaaacagtttgaagactgvcgctcgctttatgatgtgggaattaaagcttctactgatgtact 990
T L N Q L N N P E F N E D V R S L Y D G G I K L P T D V L 330
 aagcaagattagccaataccattgttctcggactcttccgatctgagtggaagcagcacttaaatccaccacaaaagtgtattca 1080
S K I S P I P L F S E L F R S D G E A A L K F P P P K V I Q 360
 agtggatcattctgcatggatgactgatgaggaatttgcagggagatgattgcccggagtgaatccacacatcatcaaggaagttctgag 1170
V D H S A W M T D E E E A R E M I A G V N P H I I K E V L S 390
 ctttccaataaagagtaaatagacagccaactctacggtgataacaccagcaaaataaccaaaagacacttggagccaaacttgggtgg 1260
F P I K S K L D S Q L Y G D N T S K I T K E H L E P N L G G 420
 agtcaactgtcgaaggggctatccagaccaacagactattcacacctgatcccatgatgcattatttccatatttgaggaaaaataatgc 1350
V T V E G A I Q T N R L F T P D H H D A L F P Y L R K I N A 450
 cactgacacaaaagcatatgctacaaggacagtccttttcttgcgaagataatggacttgaagccattggccattgagttgagtagtacc 1440
T A T K A Y A T R T V L F L Q D N G T L K P L A I E L S T P 480
 acatcccgatggtagtatttggctcctgtaagttaagtttacttgcctgcaagtgaaggtgttgaagcttcaatttggcttcttgcctaa 1530
H P D G D S F G P V S K V Y L P A S E G V E A S I H L L A K 510
 ggctttcgtagttgtaaatgactcatgctatcaccaacttgttagccatttgggtgaacactcacgctgttgttgagccattcatcatage 1620
A F V V V N D S C Y H Q L V S H W L N T H A V V E P F I I A 540
 aacaacagacatcttagtggttaccctattcataaacttttacttccacattaccgtagacacatgaacatcaacgcacttgcctag 1710
T N R H L S V V H P I H K L L L P H Y R D T M N I N A L A R 570
 aatgtcttggctcaatgcagagggattatagaatcaacattcttggggaaattatgctatgaaatgtctgctgttgtttacaagga 1800
N V L V N A E G I I E S T F L W G N Y A M E M S A V V Y K D 600
 ttgggttttccagaccaaggactacctaatgatctaataaaaagaggagtggctgttaaggatccatcttccacaggtgttgcct 1890
W V F P D Q G L P N D L I K R G V A V K D P S S P H G V R L 630
 tctgatagaggactatccttatgcttctgacggactagagatagggctgctatcaagtgcgtgggttgaagaatatgtgaatttctacta 1980
L I E D Y P X A S D G L E I W A A I K S W V E E Y V N F Y X 660
 caaatcagatgctgctattgcacaagatgccgaactccaagcattctggaagaactttagaggtgggtcatgggtgacttgaagagtg 2070
K S D A A I A Q D A E L Q A F W K E L V E V G H G D L K S A 690
 tacatggtggtttaaagatgcaaaatcgcaaggattgattgaagcctgctccatcctcatatggatagctttagcgcctacacgcagctgt 2160
T W W F K M Q N R K E L I E A C S I L I W I A S A L H A A V 720
 taatttcggacaatatccatattggaggatacattcttaaccgaccaactaaaagcaggaattcatgcctgagaagggaaaccctgagta 2250
N F G Q Y P Y G G Y I L N R P T K S R K F M P E K G T P E Y 750
 tgatgatctggctaagaactacgagaagcgtatttggaggacaatcacaccaagaatgatactctaactgacttgaccattatagaggt 2340
D D L A K N Y E K A Y L R T I T P K N D T L T D L T I I E V 780
 cttgtcaagcagcttctgatgaacaatccctggagagagaattgaaggtgatgattggactactgattcagtaacaaaagaggttt 2430
L S R H A S D E Q Y L G E R I E G D D W T T D S V P K E A F 810
 caagaggttggaaagaagttggctgaaatagaggaaaaactcactcaaggaacaacgcagagctggttggaggaatcggatggaccagt 2520
K R E G K K L A E I E E K L T Q R N N D E S L R N R Y G P V 840
 gaagatgccatcacccctacttcttctagttaggaaggattgacttgcagagggattccaaacagtagtccatctcaagaaggggc 2610
K M P X T L L Y P S S E E G L T C R G I P N S I S I * 866
 atggatatttttctgcttgaataacagatgagaagaagtttaattctctcaatctagtgatgttgttatgtatgtttatttgactg 2700
 ttttaatgttttaattacatcaataaagaactactatgtctggtcagctgtacttgtattgtatgatatacaatgcc 2778

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LLOX  MASL-----LFGRG--Q----KLKGTVILM
SL1   MFSA-----GH----KIKGTVVLM
SL2   MFSVPGVSGILNRGGGH----KIKGTVVLM
PL2   MF--PNVTGLLNKG--H----KIRGTVVLM
SL3   MLG-----GLLHRG--H----KIKGTVVLM
PL3   MFS--GVTGILNRG--H----KIKGTVVLM
SLX   MF-----GIFDKG--Q----KIKGTVVLM
BLA   MF-----GI LNKG--H----KIKGTVVLM
RL2   MLG-----GIIGGLTGNKNA-RLKGSLVLM
AL    MFG--ELRDLTGGGNETTTKVKGTVVLM
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Fig. 3. Comparison of the N-terminal sequences of plant lipoxygenases. Residues identical in all sequences are indicated by (*) in the last line, residues similar in all sequences are indicated by (.). Dashes (-) indicate gaps introduced to obtain maximal homology in the alignment.

High LOX levels, if assayed at neutral pH, even higher than in soybean, which is traditionally considered the richest source of LOX, are found in the seeds [14,15]. During germination the LOX activity in the shoot increases until 5 days after imbibition, followed by a gradual decrease (M.P. Hilbers et al., unpublished results). In order to study these LOXs at the molecular level we attempted to find the cDNA coding for such a LOX.

20 000 Clones from a λ gt10-cDNA library, made from mRNA from the shoots of 5 days old etiolated lentil seedlings [16] were screened with 2 oligonucleotide-probes complementary to a highly conserved part of legume LOX sequences (Fig. 1).

Of the 7 clones thus found the clone with the largest insert (λ lx5) was sequenced. Its 2751 bp insert was expanded towards the 5'-end using PCR on DNA from a lysate originating from 100 000 clones with one primer corresponding to the sequence of the phage near the cloning-site (either 5'-CTTTTGAGCAAGCTTAGC-CTGGTAAAG-3' or 5'-CGAGGAAGCTTATGAG-TATTTCTTCCAGGGTA-3') and the other corresponding to a part of the λ lx5 sequence (see Fig. 2). PCR artefacts can be excluded since we obtained identical sequences from different PCR experiments. The combined sequences give a 2778 bp sequence containing an open reading frame of 2598 bp which encodes for a polypeptide of 866 amino acid residues and a calculated molecular mass of 96651. Comparison with the other plant LOX sequences showed that this polypeptide has 53–72% amino acid residues identical to those of the other plant LOXs. A BLAST [17]

Table 1

Percentages of identical residues found in alignments of pairs of plant LOX sequences as created by the BESTFIT [20] program. The length of incomplete sequences is indicated in the upper row. Not included are the sequences of RL2 (53–57% identical to the other LOX's), AL (58–63% identical) and SLA (599 aa, 99% identical to SLX)

	SL1	SL2	PL2	SL3	PL3	SLX	BLA	BL3	SLB1 (741)	SLB2 (92)	PLP1 (115)	PLP1 (92)
LLOX	65	66	66	72	71	67	65	73	75	73	58	
SL1		85	79	72	71	70	69	67	69	62	65	
SL2			84	76	75	72	72	69	67	60	73	
PL2				75	75	72	72	69	68	61	73	
SL3					86	71	72	77	74	69	72	
PL3						71	69	76	76	68	73	
SLX							73	69	68	62	69	
BLA								67	68	61	62	
BL3									91	89	61	
SLB1										95	67	
SLB2												56

search against the SwissProt and GenPept databases did not result in the finding of a non-LOX sequence sharing any significant homology with our sequence, so we conclude that the sequence is indeed a LOX-sequence.

The sequence we obtained has only one nucleotide upstream from the initiation codon of the LOX-encoding open reading frame. This nucleotide was G, which might belong to the CAAAG-sequence, preceding the initiation codon in most plant LOX sequences. A comparison of the N-terminal region of the different plant LOXs (Fig. 3) shows that the sequences in this region are not very similar.

The pattern found for the N-terminal sequence of most LOXs, M-X-(S/G), with X indicating a hydrophobic residue, is also found in the LLOX sequence. Moreover it is difficult to make a good alignment with the N-terminal regions of the plant LOX sequences without aligning the putative LLOX initiation methionine with those of the other LOXs. Therefore we conclude that the sequence we have found represents the entire coding sequence.

Table 1 shows the homology of all legume LOX sequences to each other. It appears that the homology of the LLOX-sequence towards the seed LOX-sequences (65–72% identical) is lower than the homology found between those other LOXs themselves (70–86% identical). A somewhat higher homology is found towards the partial vegetative LOX sequences BL3, SLB1

Fig. 2. The LLOX cDNA sequence and its derived protein-sequence. Nucleotide 1–95 originate from the PCR, nucleotide 26–2778 from the λ lx5 clone. The underlined DNA-sequence at bp 126–150 corresponds to the primer used in the PCR, the one at bp 1574–1593 to the oligo-probes used to screen the library. Bold-faced amino acid residues were found to be identical in a multiple sequence alignment of all complete and partial LOX sequences, underlined amino acid residues were also found to be conserved in all lipoxygenases in an alignment containing the available mammalian lipoxygenase sequences [18] in addition to the plant LOX sequences. The multiple sequence alignments were created with the CLUSTALV-program [19].

and SLB2, but again this homology is less than the homology between those sequences. There is no particular high homology towards any of the other LOX sequences so it appears that we have found a sequence for a novel type of vegetative LOX. Nevertheless the sequence itself (Fig. 2) shows the usual features for plant LOXs: a long conserved region (W₅₀₇–R₅₇₀) containing 5 histidines, which are also conserved in the mammalian LOXs, the second (H₅₂₆) and the third (H₅₃₁) of these probably being ligands to iron [21] and a highly conserved region (S₇₁₄–Y₇₂₄, identical in all plant-sequences) containing another histidine, also found in the mammalian LOXs and again probably involved in iron-binding. Finally, also the lentil enzyme has the highly conserved C-terminal sequence GIPN-SISI.

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