Aalborg Universitet



Characterization of Lipoxygenases from Potato Tuber (cv. kuras)

Kristiansen, Rikke; Jørgensen, Malene; Welinder, Karen Gjesing

Publication date: 2009

Document Version Publisher's PDF, also known as Version of record

Link to publication from Aalborg University

Citation for published version (APA):

Kristiansen, R., Jørgensen, M., & Welinder, K. G. (2009). *Characterization of Lipoxygenases from Potato Tuber (cv. kuras)*. Poster presented at Danish Conference on Biotechnology and Molecular Biology, Vejle, Denmark.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- ? You may not further distribute the material or use it for any profit-making activity or commercial gain ? You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

Characterization of Lipoxygenases from Potato Tuber (cv. Kuras)

Rikke Kristiansen, Malene Jørgensen, and Karen G. Welinder Section of Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University



Lipoxygenases (Lox; EC 1.13.11.12) are region- and stereospecific monomeric dioxygenases incorporating molecular oxygen into polyunsaturated fatty acids containing a *cis,cis*-1,4pentadiene moiety. This reaction produces conjugated *cis,trans*-diene hydroperoxides (Walker *et al.*, 1996). Loxs have been found in animals, plants and bacteria (Porta and Rocha-Sosa, 2001). In plants, they are important in the biosynthetic pathway of jasmonic acid which might act as a signal molecule in wounding response. Loxs might also be involved in growth regulation and used as storage proteins. The most common substrates for plant Loxs are linoleic acid and linolenic acid (Porta and Rocha-Sosa, 2002). Plant Loxs are app. 100 kDa.

Sequence coverage of Lox isoforms

Proteolytic digestions

StLoxs were purified from potato tubers (cv. Kuras) in a number of precipitation and chromatographic steps.

In-solution digestion was carried out on MonoQ fractions. The proteins were reduced and carboxymethylated, and digested with sequencing grade modified trypsin (Promega, Madison, USA), or sequencing grade modified chymotrypsin (Boeringer Ingelheim GmbH, Ingelheim, DE). In-gel digest was performed on Lox-containing bands from SDS-gels. The proteins were reduced and carboxymethylated, and digested with sequencing grade modified trypsin (Promega, Madison, USA).

Three Lox isoforms reported in DFCI Potato Gene Index (http://compbio.dfci.harvard.edu/tgi/ cgi-bin/tgi/gimain.pl?gudb=potato) and one Kuras specific isoform were found in potato tuber (cv. Kuras). Sequence coverage of 74 % to 88 % of these isoforms was obtained by LC-ESI MS/ MS. The N-terminus was sequenced in one of the isoforms. This showed that the N-terminus of this isoform was acetylated.

Structural analysis of potato Lox

Potato Loxs can be divided into at least four classes; one expressed mostly in tuber and roots, one in leaves, one in leaves and roots, and one pathogen-induced type in leaf (Royo *et al.*, 1996; Kolomiets *et al.*, 2000; Kolomiets *et al.*, 2001). Ten potato (*Solanum tuberosum*; St) Lox isoforms have been downloaded from ExPASy and DFCI Potato Gene Index. In order to see how these Loxs cluster, a phylogenetic tree was composed from the translated cDNA sequences (figure 1) using the ClustalX2 software (http://www.clustal.org/).



Digests were analyzed by nanoflow RP-chromatography interfaced directly to an electro spray ionization Q-TOF tandem mass spectrometer (LC-ESI MS/MS) (MicroTOFQ, Bruker Daltonics, Bremen, DE). Merged MS/MS data were search by the Mascot search engine (www.matrixscience.com) towards our AAU potato protein database.

k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	10 	20 GHHDSKKVKGT D D	30 VVMMKKNALD 	40 FTDLAGSLTI S (50 DKIFEALGQKV 	60 SFQLISSVQS G	70 DPANGLQGKH 	80 . SNPAYLENFLE 	90 TLTPLAAG	10 -ETAFG S S
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	VTFDWNEEFGVPGA	 FIIKNAHINEF T T	FLKSLTLEDV	I 4 0 VPNHGKVHFVC I I I I I I I I I I I I I I I I I I I	LSU CNSWVYPSFRY	 KSDRIFFANQ	PYLPSKTPEL:	LRKYRENELLT	LRGDGTGKI	 REAWDR
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	210 IYDYDIYNDLGNPD V.	220 DEGKENVRTTLG Q Q	230 GSAEYPYPRR D	240 SGRTGRPPTRT	250 TDPKSESRIPL	260 ILSLDIYVPR L	270 DERFGHLKMS 	280 . DFLTYALKSIV	290 /QFILPELH2	30 ALFDGT
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	310 PNEFDSFEDVLRLY	320 TEGGIKLPQGPL	330 FKALTAAIPL	340 EMIRELLRTI	350	360 LVIKDSKTAW	370 RTDEEFAREM	380 . LAGVNPVIISE I	390 LQEFPPKS	40 KLDPQA E. E.
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	410 ygnqnstitaehie	420 DKLDGLTVDEA	430 MNNNKLFILN	440 IHHDVLIPYLH L	450 RRINTTTTKTY IS.	460 ASRTLLFLQD	470 MGSLKPLAIE	480 	490 	50 ASDQGV P P
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	510 ESSIWQLAKAYVAV	520 NDSGVHQLISH 	530 WLNTHAVIEP	540 ••••••••••••••••••••••••••••••••••••	550	560 PHFRDTMNIN	570 ASARQILVNA .MI 	580 . GGVLESTVFQS	590 	60 VVYKDW
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	610 VFPDQALPADLVKR	620 GVAVEDSSSPH	630 GVRLLIEDYP	640 YAVDGLEIWS	650	660 SFYYGSDEEI	670 LKDNELQAWW	680 . KELREVGHGDE	690 (KNEPWWPEN	70 MKTPQE .E .E
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	710 LIDSCTTIIWIASA	720	730 YAGYLPNRPT	740 VSRRFMPEPC	750	760 PDKAFLKTIT 	770 AQLQTLLGVS	780 . LVEILSRHTTI .I	790 DEIYLGQRES	80 SPEWTK
k1_215_StLox TC163046_StLox TC164496_StLox TC163045_StLox	810 DKEPLAAFDRFGKK 	820 CLTDIEKQIIQR	830 NGDNILTNRS	840 SGPVNAPYTLI	850	860 KGIPNSVSI				

Figure 1: Phylogenetic tree of ten potato (*Solanum* tuberosum; St) Lox sequences translated from cDNA. The four at the bottom are from leaf tissue while the rest are from tubers.

The three Lox isoforms from leaf plus leaf and root are longer at their N-termini, corresponding to chloroplast targeting signals. Tuber StLoxs are found in vacuoles, but the transport mechanism is unknown due to no known targeting signal. Tuber Loxs are more closely related to the pathogen-induced leaf Lox than the other leaf Loxs.

In all StLoxs in figure 1, the amino acids considered to be important for functionally active enzymes are conserved. These amino acids include three histidines (His⁵²¹, His⁵²⁶ and His⁷¹²; kuras k1_215 numbers) and the C-terminal isoleucine (Ile⁸⁶⁰; k1_215 number) which have been shown to bind the iron atom of the active site. A fifth active site iron ligand is a water molecule (Minor *et al.*, 1996). The substrate cavity and the iron coordination network are connected by a hydrogen bonding network composed of Gln⁵¹⁷, Gln⁷¹⁹ and Asn⁷¹⁶ (k1_215 numbers) providing a very specific cavity for substrate binding (Tomchick *et al.*, 2001). The conserved amino acids can be seen in figure 2, which is the crystal structure of soybean (*Glycine max*; Gm) Lox1 (PDB structure no. 1f8n, 1.40 Å resolution). This crystal structure was chosen because no crystal structure of a StLox exists. GmLox1 is 56 % identical to k1_215 (a Kuras specific StLox).

Figure 3: Translated cDNA from four potato tuber Lox isoforms. The grey areas correspond to peptides seen by MS/MS ion search when merging all data. The green areas correspond to amino acids found subsequently by error tolerance search.

The sequence coverage of the four potato tuber isoforms (figure 3) is as it follows; 87 % of K1_215, 81 % of TC163046, 81 % of TC164496 and 94 % of TC163045 have been sequenced. TC163045 is acetylated at the N-terminus.



Figure 2: Soybean (*Glycine max*; Gm) Lox1 (PDB structure no. 1f8n, 1.40 Å resolution) with the conserved amino acids (His⁴⁹⁹, His⁵⁰⁴, His⁶⁹⁰, Ile⁸³⁹, Asn⁶⁹⁴, Gln⁴⁹⁵ and Gln⁶⁹⁷ GmLox1 numbers). Left: GmLox1 with the active site in the middle. Right: Close up at the active site. The histidines are shown in red, the isoleucine in yellow, the asparagine in green, and the glutamines in cyan.



Kolomiets *et al.*, 2000: Kolomiets, M. V., Hannapel, D. J., and Gladon, R. J. (2000), *A Leaf Lipoxygenase of Potato Induced Specifically by Pathogen Infection*, Plant Physiol. **124**, pp. 1121-1130

Kolomiets *et al.*, 2001: Kolomiets, M. V., Hannapel, D. J., Chen, H., Tymeson, M., and Gladon, R. J. (2001), *Lipoxygenase is Involved in the Control of Potato Tuber Development*, The Plant Cell **13**, pp. 613-626.

Minor *et al.*, 1996: Minor, W., Steczko, J., Stec, B., Otwinowski, Z., Bolin, J. T., Walter, R., and Axelrod, B. (1996), *Crystal Structure of Soybean Lipoxygenase L-1 at 1.4 Å Resolution*, Biochemistry, **35**, pp. 10687-10701

Porta and Rocha-Sosa, 2001: Porta, H., and Rocha-Sosa, M. (2001), *Lipoxygenases in bacteria: a horizontal transfer event?*, Microbiology **147**, pp. 3199-3200

Porta and Rocha-Sosa, 2002: Porta, H., and Rocha-Sosa, M. (2002), *Plant Lipoxygenases. Physiological and Molecular Features*, Plant Physiology **130**, pp. 15-21

Royo *et al.*, 1996: Royo, J., Vancanney G., Pérez, A. G., Sanz C., Störmann, K., Rosahl, S., and Sánchez-Serrano, J. (1996), *Characterization of Three Potato Lipoxygenases with Distinct Enzymatic Activities and Different Organ-specific and Wound-regulated Expression Patterns*, The Journal of Biological Chemistry **271**, pp. 21012-21019

Tomchick *et al.*, 2001: Tomchick, D. R., Phan, P., Cymborowski, M., Minor, W., and Holman, T. R. (2001), *Structural and Functional Characterization of Second-Coordination Sphere Mutants of Soybean Lipoxygenase-1*, Biochemistry **40**, pp. 7509-7517