Characterization of Lipoxygenases from Potato Tuber (cv. kuras)

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Characterization of Lipoxygenases from Potato Tuber (cv. Kuras)

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Introduction and Conclusion

Lipoxygenases (Lox; EC 1.13.11.12) are region- and stereospecific monomeric dioxygenases incorporating molecular oxygen into polyunsaturated fatty acids containing a *cis,cis*-1,4-pentadiene 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.

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/).

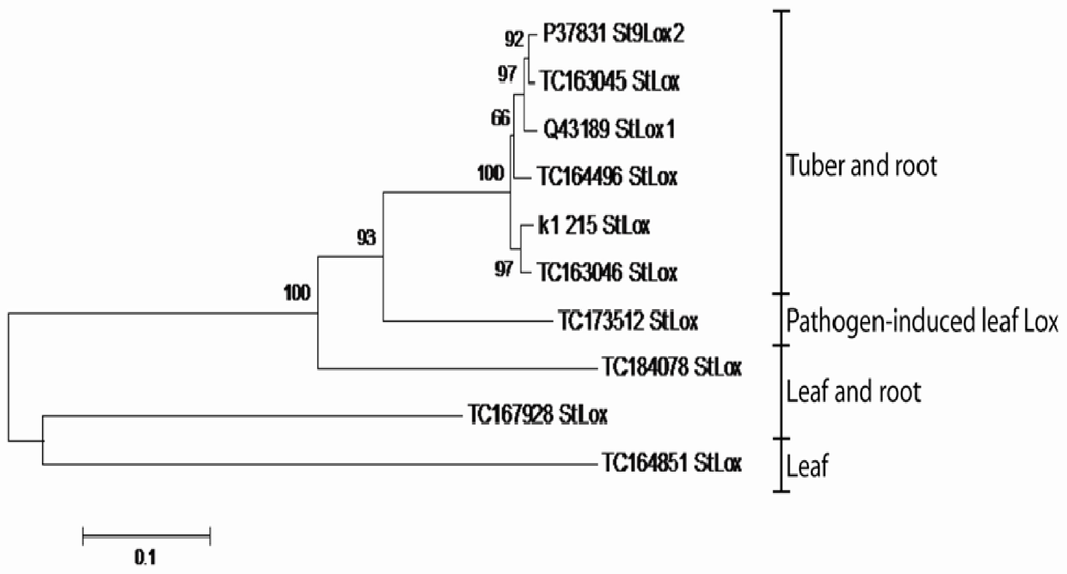

Figure 1: Phylogenetic tree of ten potato (*Solanum* tuberosum; St) Lox sequences translated from cDNA. The four at the bottom are from leaf tissue while the rest are from tubers.

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 (His^{521}, His^{526} and His^{712}; kuras k1_215 numbers) and the C-terminal isoleucine (Ile^{860}; 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 Gln^{517}, Gln^{719} and Asn^{716} (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).

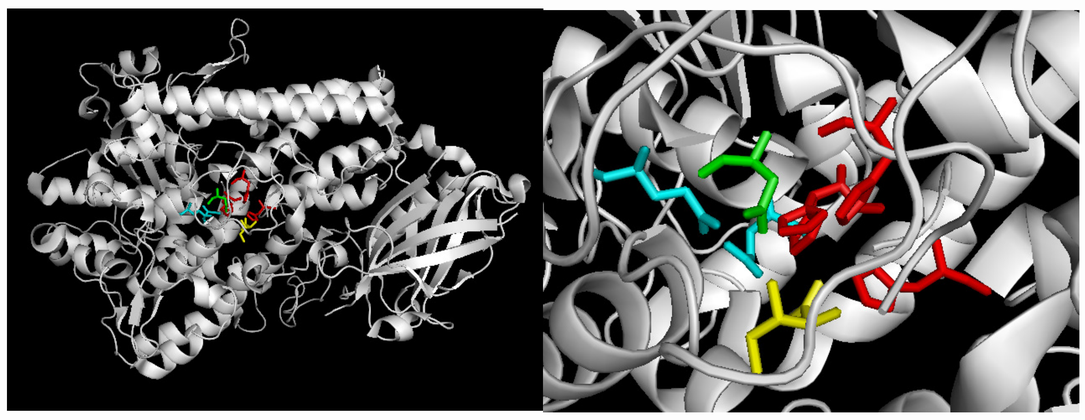

Figure 2: Soybean (*Glycine max*; Gm) Lox1 (PDB structure no. 1f8n, 1.40 Å resolution) with the conserved amino acids (His^{499}, His^{504}, His^{690}, Ile^{839}, Asn^{694}, Gln^{495} and Gln^{697} GmLox1 numbers). Left: GmLox1 with the active site in the middle. Right: Close up at the active site. The histidines are shown in red, the isoleucine in yellow, the asparagine in green, and the glutamines in cyan.

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).

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.

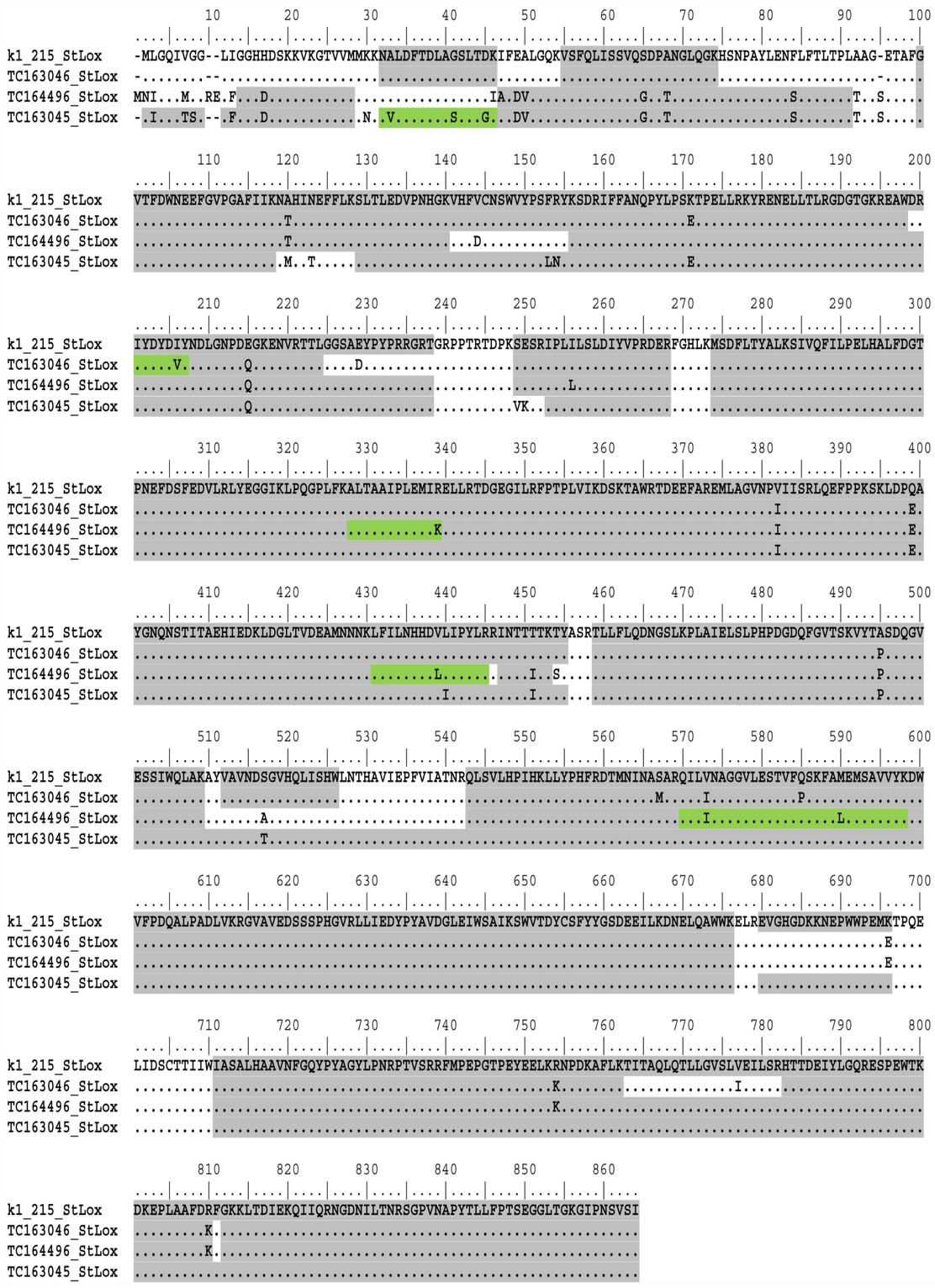

Figure 3: Translated cDNA from four potato tuber Lox isoforms. The grey areas correspond to peptides seen by MS/MS ion search when merging all data. The green areas correspond to amino acids found subsequently by error tolerance search.

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.

References

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