Characterization of Lipoxygenases from Potato Tuber (cv. kuras)

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Lipoxygenases (Lox; EC 1.13.11.12) are region- and stereospecific monomeric dioxygenases incorporating molecular oxygen into polyunsaturated fatty acids containing a cis-1,4-pentadiene moiety. This reaction produces conjugated cis-1,4-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 jasmonic pathway which might act as a signal molecule in wound 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.

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). Potato 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 ClustalW software (http://www.clustal.org/).

Figure 1: Phylogenetic tree of ten potato (Solanum tuberosum) Lox isoforms sequenced from cDNA. The four at the bottom are from leaf tissue while the rest are from tubers.

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 (His495, His499 and His690) kuras k1_215 numbers) and the C-terminal isoleucine (Ile694, Ile697 and Gln712, Gln716 and Asn719) with the conserved amino acids (His495, Gln499 and Asn504, Gln690 and Gln697, Gln712 and Asn719) 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 (His495, Gln499, His499, Asn504, Gln690 and Gln697, Gln712 and Asn719) numbers) and the C-terminal isoleucine (Ile694, Ile697 and Gln712, Gln716 and Asn719) 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 glutaminines in cyan.

Proteolytic digests

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 sequence grade modified chymotrypsin (Boehringer Ingelheim GmbH, Ingelheim, DE). In-gel digest was performed on Loxs-containing bands from SDS-gels. The proteins were reduced and carboxymethylated, and digested with sequencing grade modified trypsin (Promega, Madison, USA).

Digests were analysed by nanoflow RP-chromatography interfaced directly to an electrospray ionization Q-TOP 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.

Sequence coverage of Lox isoforms

The sequence coverage of the four potato tuber isoforms (figure 3) is as it follows: 87 % of k1_215, 81 % of TC163846, 81 % of TC164496 and 94 % of TC163045 have been sequenced. TC163045 is acetylated at the N-terminus.

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.

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References