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):
Introduction and Conclusion

Lipoxygenases (Lox; EC 1.13.11.12) are region- and stereospecific monomeric dienesynases incorporating molecular oxygen into polyunsaturated fatty acids containing a cis-1,4- pentadienone 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 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 digests

StLox 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 then analyzed by MALDI QTOF (Bruker Daltonics, Bremen, DE) or ESI MS/MS tandem mass spectrometer (LCQ Deca, Bruker Daltonics, Bremen, DE). Merged MS/MS data were searched by the Mascot search engine (www.matrixscience.com) towards our AAU potato protein database.

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). Tomato Loxs (Solanum tubersum), St Loxs 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, compared 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 (His104, His105 and His212) kuras k1_215 numbers) and the C-terminal isoleucine (Ile839, kuras k1_215 numbers) 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 Gln576, Gln578 and Asn764 (k1_215 numbers) providing a very specific cavity for substrate binding (Toschick et al., 2001).

Figure 2: Soybean (Glycine max; Gm) Lox1 (PDB structure no. 1l0h, 1.40 Å resolution) with the conserved amino acids (His104, His105, His212, Asn214, Gln276 and Gln578 GmLox1 numbers). Left: GmLox1 with the active site in the middle. Right: Close up of the active site. The histidines are shown in red, the isoleucine in yellow, the asparagine in green, and the glutamines in cyan.

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 Lox 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.

References


