Note

Partial Amino Acid Sequences of Soybean Lipoxygenase L-6 Isolated from Cotyledons

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Lipoxygenases catalyze the addition of molecular oxygen to fatty acids containing cis,cis-pentadiene structures. Recent studies of plant lipoxygenases have shown that they are involved in the biosynthesis of physiologically active compounds such as the plant growth regulator, jasmonate, and antifungal compounds. The biosynthesis of compounds responsible for flavor has been studied in soybean cultivars that lack certain lipoxygenase isozymes, and some of the byproducts of soybean processing which are responsible for undesirable food flavors appear to result in part from lipoxygenase activity.

Three lipoxygenases, L-1, L-2, and L-3, have been purified from dry soybean seeds and studied extensively, and the molecular cloning of these three lipoxygenases has been completed. Several other lipoxygenases, distinct from the three seed isozymes, have also been identified in the hypocotyl/radicle and leaf tissues of soybean. Recently Kato et al. characterized three new soybean lipoxygenases, L-4, L-5, and L-6, which appear in the cotyledons after seed germination. Partial amino acid sequencing of proteolytic peptides from the L-4 enzyme showed that it shares homology with L-1, L-2, and L-3, but is not identical to any of these enzymes. Thus, it appears that the gene encoding lipoxygenase L-4 is different from those encoding L-1, L-2, and L-3 and is specifically expressed in cotyledons.

Consistent with the identification of these three new lipoxygenases in cotyledons, cDNAs encoding new lipoxygenases were found in a library made from 5-day-old soybean cotyledons, suggesting that the lipoxygenases are newly synthesized in cotyledons. In continuing the characterization and molecular cloning of the cotyledon lipoxygenases, we partially analyzed the amino acid sequence of lipoxygenase L-6 in order to find if this enzyme is derived from a gene that differs from the previously identified lipoxygenase genes or if it is a modified form of a preexisting lipoxygenase. We purified the L-6 enzyme from 5-day-old cotyledons of a variant soybean cultivar, Kanto 101, which contains lipoxygenase L-1, but not the L-2 or L-3 isozymes.

The L-6 enzyme was purified by ammonium sulfate fractionation, DEAE-Toyopearl chromatography, CM-Toyopearl chromatography, and Mono S chromatography as previously described. The purified protein (100 μg) was digested with 1 μg of lysylendopeptidase (Wako Pure Chemical Industries Ltd, Japan) in 0.1 M TrisCl, pH 8.0, at 37°C for 6 hr. After lyophilization, the resulting peptides were dissolved in 0.1 M of 0.1% trifluoroacetic acid and treated by reverse phase HPLC using an ODS column (4.6 × 250 mm, Biofine RPC-SC18, Japan Spectroscopic Co., Ltd.). The peptides were eluted with linear gradients of 0–80% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min for 80 min and monitored at 220 nm. The separated peptides were analyzed using an automated amino acid sequencer (ABI).

A total of 24 amino acid residues were identified in 5 peptides. We compared the amino acid sequences of these peptides with the sequences of the previously characterized soybean lipoxygenases. These 5 peptides are all partially homologous but not identical to regions of lipoxygenases L-1, L-2, and L-3 (Fig. 1). This indicates that the gene encoding lipoxygenase L-6 is distinct from genes encoding these three lipoxygenases and that it is probably expressed specifically in cotyledons.

We also aligned the partial amino acid sequences of the 5 peptides to amino acid sequences from soybean lipoxygenase L-4 as deduced from the nucleotide sequence of the lipoxygenase L-4 gene (unpublished data). This alignment indicates that 17 amino acid residues are identical, while the remaining residues differ. We conclude that the gene encoding lipoxygenase L-6 distinct from the gene encoding lipoxygenase L-4.

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References


Fig. 1. Comparison of the Amino Acid Sequences of Peptides of Soybean Lipoxygenase L-6 with Previously Identified Lipoxygenases L-1, L-2, and L-3.

The amino acid sequences of 5 peptides from soybean lipoxygenase L-6 are compared with sequences from soybean lipoxygenases L-1, L-2, and L-3. The position (amino acid number) of the amino terminal residue of each peptide is written in brackets with respect to the initial methionine residue of the corresponding lipoxygenase. Amino acid residues identical to those in lipoxygenase L-6 are indicated by a colon (:). Unidentified amino acid residues are indicated by a question mark (?).