Cloning and Sequencing of a Full-Length cDNA Coding for sn-Glycerol-3-Phosphate Acyltransferase from Phaseolus vulgaris

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One of the possible factors thought to control chilling tolerance in plants is the lipid composition of plastidial membranes. In particular, a high proportion of htm-PG has been correlated to chilling sensitivity (Murata, 1983). The proportion of htm-PG synthesized in plastids (Mudd et al., 1987) can be attributed to the properties of a single enzyme, GPAT (EC 2.3.1.15). This acyl transferase initiates glycerolipid synthesis by catalyzing the acylation of the sn-1 position of sn-glycerol-3-phosphate, producing sn-1-acyl-glycerol-3-phosphate. In chilling-tolerant plants such as pea, spinach, and Arabidopsis, the acyl transferase selects oleic acid (18:1) from a mixture of acyl-ACPs containing mainly 18:1 and palmitic acid (16:0) (Frentzen et al., 1983). In contrast, in chilling-sensitive plants such as cucumber, squash, and French bean the enzyme accepts all acyl groups present in the acyl-ACP pool and thus introduces a relatively high proportion of saturated fatty acids into the sn-1 position. Subsequent to incorporation the saturated fatty acids cannot be desaturated and result in htm-PG.

The fatty acid composition of plastidial membranes can be altered by expression of heterologous GPAT genes (Murata et al., 1992; Wolter et al., 1992). As a first step in genetic engineering of the fatty acid composition of plastidial PG involving replacement of the endogenous unselective GPAT by a selective one, a cDNA for the plastidial GPAT from Phaseolus vulgaris was isolated. For this purpose degenerate oligonucleotides were deduced from the known GPAT sequences of Arabidopsis, pea, cucumber, and squash and used as primers in a PCR experiment with Phaseolus leaf cDNA as template. The amplified PCR fragment was cloned and partially sequenced. To find a full-length clone we made a cDNA library from leaves and screened the library with the PCR-generated digoxigenin-labeled fragment (Boehringer). Eight positive clones were partially sequenced using the Taq Dye Primer Cycle Sequencing (Applied Biosystems, Foster City, CA). The longest clone was fully sequenced on both strands.

Method of Identification: Comparison with the deduced amino acid sequences of the GPATs from squash, cucumber, Arabidopsis, and pea.

Features of cDNA:
The clone is 1919 nucleotides long with an open reading frame from position 7 to 1392 followed by a 3' untranslated region and a poly(A) stretch of 23 residues. (G+C) content, 42%.

Features of Deduced Amino Acid Sequence:
The open reading frame codes for a polypeptide of 461 amino acids. The putative mature protein shows 70.4% homology with the squash GPAT (Ishizaki et al., 1988), 68.8% with that of cucumber (Johnson et al., 1992), 67.2% with that of Arabidopsis (Nishida et al., 1993), and 83.2% with that of pea (Weber et al., 1991).

Antibodies:
Antibodies against GPAT from P. vulgaris have not been generated.

Table I. Characteristics of the chloroplast GPAT gene from P. vulgaris

| Organism: | French bean (Phaseolus vulgaris L. cv Annabel). |
| Source: | DNA library from leaf RNA in a Zap (Stratagene). |
| Method of Isolation: | DNA hybridization screening of 2.15 x 10^6 recombinant phages with PCR-generated digoxigenin-labeled fragments (Boehringer). Eight positive clones were partially sequenced using the Taq Dye Primer Cycle Sequencing (Applied Biosystems, Foster City, CA). The longest clone was fully sequenced on both strands. |
| Method of Identification: | Comparison with the deduced amino acid sequences of the GPATs from squash, cucumber, Arabidopsis, and pea. |
| Features of cDNA: | The clone is 1919 nucleotides long with an open reading frame from position 7 to 1392 followed by a 3' untranslated region and a poly(A) stretch of 23 residues. (G+C) content, 42%. |

Abbreviations: ACP, acyl-carrier protein; GPAT, acyl-ACP:sn-glycerol-3-phosphate acyltransferase; htm-PG, high-temperature melting point phosphatidylglycerol; PG, phosphatidylglycerol.

1 The financial support of GTZ (Program No. 91.7860.9-01-114) is acknowledged. This article is based on a doctoral study by M.F. in the Faculty of Biology, University of Hamburg.

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stretch of 23 residues and contains an 1383-bp open reading frame capable of coding for a putative polypeptide with 461 amino acids and a 536-bp 3’ noncoding region (Table I). The clone is thought to code for an N-terminal transit peptide of 96 residues (the deduced amino acid sequence is numbered beginning from the first ATG codon of the open reading frame) and the mature GPAT with 365 amino acids. Because a stop codon is present in front of the first ATG of the open reading frame, we conclude that this clone represents the complete coding region. The molecular mass of the unprocessed polypeptide was calculated to be approximately 50 kDa.

Received September 6, 1994; accepted October 9, 1994.

LITERATURE CITED


