

Plant Gene Register

Nucleotide Sequence of a *Clarkia breweri* cDNA Clone of *Ipi1*, a Gene Encoding Isopentenyl Pyrophosphate Isomerase¹

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IPP, a compound derived from the mevalonic acid pathway, is part of several important biochemical pathways in plants. It is a starting point for the synthesis of certain hormones. In addition, IPP and its isomer DMAPP are the building blocks for the numerous terpenoid compounds, e.g. carotenoids, sterols, and rubber, to name a few. Our group has been studying the synthesis of several monoterpenoid floral scent compounds in *Clarkia breweri*, an annual plant native to California (Pichersky et al., 1994). Monoterpenes are formed by the condensation of one IPP molecule with one DMAPP molecule. DMAPP and IPP are interconvertible; this reaction is catalyzed by the enzyme IPI (Anderson et al., 1989).

IPI has been investigated in animals (Bruenger et al., 1986), plants (Spurgeon et al., 1984; Dogbo and Camara, 1987; Albrecht and Sandman, 1994), and microorganisms (Anderson et al., 1989). A gene encoding IPI has been isolated from yeast (Anderson et al., 1989). Recently, an Arabidopsis cDNA clone randomly chosen for partial sequencing in the Arabidopsis genome project was found to have limited, but significant, sequence similarity to the yeast *Ipi* gene. We have used this Arabidopsis cDNA clone to identify several cDNA clones from a *Clarkia breweri* cDNA library prepared from poly(A⁺) mRNA isolated from petals and stigmata of unopened buds and just-opened flowers (Table I).

Several cDNAs were obtained, and one clone was chosen for further characterization. Its nucleotide sequence displays an open reading frame with 287 codons. The encoded protein has a calculated molecular mass of 32,970 D and is highly ionic, with charged residues making up 27% of the protein. The overall sequence identity with the yeast *Ipi*-encoded protein is slightly less than 50%. The sequence similarity is concentrated in the middle of the protein, with a short region (residues Asn¹⁰⁴–Leu¹⁴⁶ in the *Clarkia* protein) showing 81% identity with the corresponding yeast sequence, and is negligible at the N and C termini. The overall identity of the *Clarkia* IPI protein with the complete Arabidopsis sequence that we obtained (data not shown) is much higher at 90%. However, the similarity between the

Table 1. Characteristics of *Ipi1* from *C. breweri*

Organism:	<i>Clarkia breweri</i> (Onagraceae).
Location:	Nuclear gene.
Techniques:	The cDNA was isolated from a λZap II cDNA library prepared from poly(A ⁺) mRNA isolated from petals and stigmata of unopened buds and just-opened flowers of <i>C. breweri</i> . The library was screened with a partial <i>Ipi</i> cDNA clone of <i>Arabidopsis thaliana</i> (clone IG2T7P obtained from the Arabidopsis Biological Resource Center, The Ohio State University, Columbus, OH). The <i>Clarkia</i> clone was sequenced on both strands by the dideoxy method. Specific and universal primers were used.
Methods of Identification:	The clone was originally identified as an <i>Ipi</i> sequence by its sequence similarity to yeast <i>Ipi</i> . The <i>Clarkia Ipi1</i> clone was subcloned into the pET-8c expression vector and the protein produced in <i>E. coli</i> (see text) was assayed and shown to possess IPI activity.
Features of Amino Acid Sequence:	The cDNA has an open reading frame of 287 codons, with a calculated molecular mass of 32,970 D. A putative transit peptide is found at the N terminus. The <i>Clarkia</i> IPI protein sequence is 90% identical with the Arabidopsis sequence and less than 50% identical with yeast IPI.
Subcellular Location:	Presumed plastidic.

two plant sequences begins at codon 60 (numbering refers to the *Clarkia* gene), an Asp residue, and the corresponding peptide sequences preceding this residue in the two proteins show no similarity to each other. This nonconserved N-terminal sequence of 59 residues is rich in hydroxylated, small hydrophobic, and positively charged residues, and it therefore resembles a plastidic transit peptide (Keegstra et al., 1989). The presence of a transit peptide is consistent with the reported plastidic localization of the enzyme from tomato leaf tissue (Maudinas et al., 1977).

To check for the essentiality of the N-terminal peptide segment for enzyme activity, we subcloned a DNA fragment that begins at an *Nco*I site that falls on a Met codon at position 55 and ends past the stop codon of the *Clarkia Ipi1* open reading frame, into the pET-T7 expression system (Studier et al., 1990). The protein expressed in *Escherichia coli* was thus an N-terminal truncation of *Clarkia* IPI that began with Met⁵⁵. An enzyme activity assay of lysates prepared from *E. coli* cultures carrying this construct and

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Abbreviations: DMAPP, dimethylallyl PPI; IPI, isopentenyl PPI isomerase; IPP, isopentenyl PPI.

induced with isopropylthio- β -galactoside showed considerable IPI activity, whereas noninduced *E. coli* cultures or induced *E. coli* cultures not carrying this recombinant plasmid did not. These results establish that the *Clarkia Ipi1* gene encodes IPI and that the first 54 residues of the protein are not essential for enzyme activity. This region may be a plastid transit peptide, which is cleaved after import. The mature protein would then be approximately 27 kD. The reported estimated molecular mass for plant IPI is 33.5 to 34 kD (Spurgeon et al., 1984; Dogbo and Camara, 1987), which is almost identical with the calculated molecular mass of the protein encoded by the entire open reading frame of *C. breweri Ipi1* and thus argues against processing. However, it should be noted that the yeast IPI protein is predicted from the gene sequence to have a molecular mass of 33 kD but it migrates on SDS-PAGE as a 40-kD protein (Anderson et al., 1989), and it is thus possible that the plant IPI molecular mass may similarly be overestimated.

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LITERATURE CITED

- Albrecht M, Sandman G** (1994) Light-stimulated biosynthesis during transformation of maize etioplasts is regulated by increased activity of isopentenyl pyrophosphate isomerase. *Plant Physiol* **105**: 529–534
- Anderson MS, Muehlbacher M, Street IP, Proffitt J, Poulter CD** (1989) Isopentenyl diphosphate: dimethylallyl diphosphate isomerase. *J Biol Chem* **264**: 19169–19175
- Bruenger E, Chayet L, Rilling HC** (1986) Isopentenyl pyrophosphate isomerase: isolation from *Claviceps* sp. SD58 and comparison to the mammalian enzyme. *Arch Biochem Biophys* **248**: 620–625
- Dogbo O, Camara B** (1987) Purification of isopentenyl pyrophosphate isomerase and geranylgeranyl pyrophosphate synthase from Capsicum chromoplasts by affinity chromatography. *Biochim Biophys Acta* **920**: 140–148
- Keegstra K, Olsen LJ, Theg SM** (1989) Chloroplastic precursors and their transport across the envelope membranes. *Annu Rev Plant Physiol Plant Mol Biol* **40**: 471–501
- Maudinas B, Bucholtz ML, Papastephanou C, Katiyar SS, Briedis AV, Porter JW** (1977) The partial purification and properties of a phytoene synthesizing enzyme system. *Arch Biochem Biophys* **180**: 354–362
- Pichersky E, Raguso RA, Lewinsohn E, Croteau R** (1994) Floral scent production in *Clarkia* (Onagraceae). I. Localization and developmental modulation of monoterpene emission and linalool synthase activity. *Plant Physiol* **106**: 1533–1540
- Spurgeon SL, Sathayamoorthy N, Porter JW** (1984) Isopentenyl pyrophosphate isomerase and prenyltransferase from tomato fruit plastids. *Arch Biochem Biophys* **230**: 446–454
- Studier WF, Rosenberg AH, Dunn JJ** (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol* **185**: 60–89