ATCase carries out the first committed step in de novo pyrimidine nucleotide biosynthesis in plants, and its activity is regulated via classical end-product inhibition by UMP or its products (Lovatt and Cheng, 1984). As an important first step in understanding the mechanisms by which plant ATCase expression is regulated, we have cloned a pea (Pisum sativum L.) ATCase gene. This appears to be the first plant ATCase gene to have been isolated. With the exception of a recent report concerning the cloning of an alfalfa carbamoyl-P synthetase gene encoding a protein similar to the Gln-utilizing pyrimidine pathway carbamoyl-P synthetase II found in eukaryotic cells (Maley et al., 1992), no other plant genes encoding enzymes of the pyrimidine pathway appear to have been isolated.

The pea leaf ATCase was isolated by functional complementation of uracil auxotrophy in Escherichia coli strain TB-2, in which part or all of the pyrB operon encoding the catalytic and regulatory subunits of ATCase has been deleted (Roof et al., 1982). We have isolated cDNAs representing at least two distinct pyrB genes in pea, as determined by restriction mapping and sequencing (Table I). We do not presently know whether these genes are allelic. The first gene, described here and designated pyrB1 (a second pea ATCase gene, pyrB2, is presently being characterized), has been completely sequenced and exhibits a single open reading frame of 386 amino acids. Residues 1 to 55 exhibit features characteristic of a chloroplast transit peptide, consistent with reports that ATCase is a chloroplast-localized enzyme (Doremus and Jagendorf, 1985). Mature pea ATCase is labeled by wheat germ ATCase antibody and exhibits an M, of 37,000 on immunoblots, similar to that of the catalytic subunits of several bacterial ATCases, and does not appear to be part of a multifunctional complex like the CAD protein (Davidson et al., 1990).

A comparison of the primary structures of prokaryotic and eukaryotic ATCases with that of pea indicates that several domains are highly conserved, especially those comprising the carbamoyl-P and L-Asp-binding sites (Kantrowitz and Lipscomb, 1988). In addition, the last 50 residues at the C-terminal end of most other eukaryotic ATCases are highly homologous to that of the pea enzyme. Pea ATCase has a putative pyrimidine-binding site that strongly resembles part of the pyrimidine-binding site on the regulatory subunit of the E. coli ATCase (Goruaux et al., 1990). Pea ATCase also exhibits a putative L-Orn-binding site that is similar to the Orn-binding site of eukaryotic Orn transcarbamoylases, supporting the theory that ATCases and Orn transcarbamoylases may have arisen by evolutionary divergence from a common ancestral carbamoyltransferase (Huygen et al., 1987). Squash ATCase activity has also been reported to be stimulated by Orn (Lovatt and Cheng, 1984).

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**Table I. Characteristics of ATCase cDNA from pea**

| Location in Genome, Gene Name: | Nuclear genome; pyrB1. |
| Gene Product, Pathway: | ATCase (EC 2.1.3.2); pyrimidine nucleotide biosynthesis. |
| Clone Type, Designation: | cDNA, full-length; pATC57. |
| Source: | Pea leaf cDNA library in λZAP II (Williamson and Slocum, 1992). |
| Techniques: | Clones isolated by functional complementation of deleted pyrB (ATCase) gene in E. coli strain TB-2 (Roof et al., 1982) in uracil-minus medium; plasmid insert sequenced (both strands) using the dideoxy sequencing method. |
| Method of Identification: | E. coli TB-2 mutant transformed with pATC57 exhibits ATCase activity and uracil prototrophy; sequence comparisons with known ATCases (e.g. 38% identical and 22% conserved amino acids with E. coli ATCase catalytic subunit [Hoover et al., 1983]). |

**Expression Characteristics:**

Low abundance 1.6-kb transcript in pea leaf tissues.

**Features of cDNA Structure:**

Insert of 1593 bp. Open reading frame: translation start at nucleotide 175 and stop at nucleotide 1333.

**Features of Deduced Protein:**


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The GenBank accession number for the sequence reported in this article is M96981.

LITERATURE CITED


Goruaux JE, Stevens RC, Lipscomb WN (1990) Crystal structures of aspartate carbamoyltransferase ligated with phosphonoacetamide, malonate, and CTP or ATP at 2.8 Å resolution and neutral pH. Biochemistry 29: 7702-7715


