Molecular Cloning of the Biotinylated Subunit of 3-Methylcrotonyl-Coenzyme A Carboxylase of *Arabidopsis thaliana*1

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Biotin-dependent carboxylases are ubiquitous enzymes necessary for a variety of anabolic and catabolic pathways. In plants, six biotinylated polypeptides have been detected (Wurtele and Nikolau, 1990), and four biotin-dependent carboxylase enzymes have been demonstrated: acetyl-CoA carboxylase (Egli et al., 1993, and refs. therein), MCCase, pyruvate carboxylase, and propionyl-CoA carboxylase (Wurtele and Nikolau, 1990). In mammals, MCCase is an enzyme of Leu degradation (Lau et al., 1980). The physiological function of MCCase in plants may be similar.

We have isolated a full-length *Arabidopsis* cDNA clone of MCCase (BP-1), based on its ability to direct the synthesis of a biotinylated protein in *Escherichia coli*. This biotinylated protein presented epitopes that were recognized by antibodies directed against the biotinylated subunit of MCCase from tomato (Wang et al., 1994). The deduced amino acid sequence derived from the nucleotide sequence of BP-1 showed high identity with the sequences of the biotinylated subunit of MCCase from tomato (Wang et al., 1994) and soybean (Song et al., 1994). These characterizations identify BP-1 as a clone coding for the biotinylated subunit of MCCase from *Arabidopsis* (Table I).

Consistent with the mitochondrial location of MCCase in animals (Lau et al., 1980) and plants (Alban et al., 1993), the deduced amino terminus of the cDNA sequence has the characteristics of a mitochondrial transit peptide. A consensus mitochondrial cleavage site, RYIS, is found at residues 23 to 26 (von Heijne, 1992). If we assume that the RY/S sequence is the junction between the transit peptide and the mature protein, then the mature protein of 690 amino acids has a calculated *M*<sub>r</sub> of 75,005.

Based on sequence identity with biotin-containing carboxylases, the amino-terminal two-thirds of the MCCase biotinylated subunit represents the biotin carboxylase domain, and the carboxyl-terminal one-third of the MCCase polypeptide contains the biotin carboxyl-carrier domain.

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**Table I. Characteristics of the *Arabidopsis* MCCase biotinylated subunit cDNA clone**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td><em>Arabidopsis thaliana</em> ecotype Columbia.</td>
</tr>
<tr>
<td>cDNA</td>
<td>BP-1 encoding the biotinylated subunit of MCCase (EC 6.1.4.1) from <em>A. thaliana</em>.</td>
</tr>
<tr>
<td>Cloning</td>
<td>A cDNA library of poly(A) enriched RNA from <em>Arabidopsis</em> seedlings in the phage AYES (Elledge et al., 1991) was screened with alkaline phosphatase-labeled ABC reagent (biotin-avidin complex; Vector Laboratories, Burlingame, CA).</td>
</tr>
<tr>
<td>Sequencing</td>
<td>Restriction fragments of the cDNA were subcloned into phagemid pBluescript (SK+ or KS+, Stratagene) and single- or double-stranded DNA was sequenced with universal and reverse primers by dideoxy chain termination technique.</td>
</tr>
<tr>
<td>Method of Identification</td>
<td>BP-1 directs the production of a biotinylated polypeptide that presents antigens recognized by tomato MCCase antibody.</td>
</tr>
</tbody>
</table>

Features of the cDNA:

- The *cDNA* is 2394 nucleotides in length and has an open reading frame of 2145 nucleotides from position 28 to 2173.

Features of the Deduced Protein:

- 715 amino acids encoding a precursor with calculated *M*<sub>r</sub> 78,133.
- Putative mitochondrial transit sequence amino acids 1 to 25. The resulting mature protein (690 amino acids) would have a calculated *M*<sub>r</sub> 75,005.
- Biotinylation site near the carboxyl terminus (amino acids 678-680).
biotin carboxylase domains of these proteins may be because the function of the biotin carboxyl-carrier domain is more structural than catalytic (i.e., it serves as a site for the attachment of biotin). Indeed, site-directed mutagenesis of the biotin carboxyl-carrier domain of transcarboxylase indicates that recognition of the biotinylation site by biotin ligase may depend on secondary and tertiary structures (Murtif and Samols, 1987).

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The GenBank accession number for the sequence reported in this article is U12536.

**LITERATURE CITED**


Elledge S, Mulligan J, Ramer S, Spottswood M, Davis R (1991) \textsc{ayes}: a multifunctional \textsc{cDNA} expression vector for the isolation of genes by complementation of yeast and \textit{Escherichia coli} mutations. Proc Natl Acad Sci USA 88: 1731–1735


