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Plant Gene Register

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

Lisa M. Weaver, Laurie Lebrun, Amie Franklin, Laiqiang Huang, Neil Hoffman, Eve Syrkin Wurtele, and Basil J. Nikolau*

Department of Biochemistry and Biophysics (L.M.W., L.L., B.J.N.), Department of Botany (E.S.W.), Iowa State University, Ames, Iowa 50011; and Department of Plant Biology, Carnegie Institution, Stanford, California 94305 (A.F., L.H., N.H.)

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 RYI/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_r 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. The biotin carboxylase domain is the most conserved region of the MCCase protein. This region contains a putative nucleotide-binding site, ^{199}GGGGKG, which fits the nucleotide-binding consensus sequence GXGXXG (Moller and Amons, 1985) and probably represents the ATP-binding site. The biotin carboxyl-carrier domain of biotin-containing enzymes share fewer identities; the sequences immediately surrounding the biotinylation site, ^{678}MKM, comprise the most conserved region of this domain. The lower sequence identity between the biotin carboxyl-carrier domains of biotinylated proteins compared to that between

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

Organism:	*Arabidopsis thaliana* ecotype Columbia.
cDNA:	BP-1 encoding the biotinylated subunit of MCCase (EC 6.1.4.1) from *A. thaliana*.
Cloning:	A cDNA library of poly(A) enriched RNA from *Arabidopsis* seedlings in the phage λYES (Elledge et al., 1991) was screened with alkaline phosphatase-labeled ABC reagent (biotin-avidin complex; Vector Laboratories, Burlingame, CA).
Sequencing:	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.
Method of Identification:	BP-1 directs the production of a biotinylated polypeptide that presents antigens recognized by tomato MCCase antibody.
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_r 78,133. Putative mitochondrial transit sequence amino acids 1 to 25. The resulting mature protein (690 amino acids) would have a calculated M_r 75,005. Biotinylation site near the carboxyl terminus (amino acids 678–680).

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* Corresponding author; e-mail dimmas@iastate.edu; fax 1–515–294–0453.

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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