in ACL activity. The presence of a severe phenotype in plants with only a slight reduction in ACL activity provides a glimpse into the importance of ACL in plant metabolism.

**Conclusion**

ACL encoded by the ACL-A and ACL-B cDNAs is cytosolic. Transgenic *Arabidopsis* with reduced levels of ACL activity have reduced cuticular waxes and seed-coat flavonoids, indicating that ACL plays an essential role in the production of cytosolic acetyl-CoA to support the synthesis of waxes and seed-coat flavonoids, indicating that ACL is an integral component of plant metabolism and development.

This work was supported by the Consortium for Plant Biotechnology, U.S. Department of Agriculture-National Research Initiative (competitive grant no. 97-01912), the Monsanto Company and a Herman Frasch award (to E. S. W.).

**References**


Received 19 June 2000

---

**Biotin carboxyl carrier protein isoforms in Brassicaceae oilseeds**

J. J. Thelen*, S. Mekhedov and J. B. Ohlrogge

Department of Botany and Plant Physiology, Michigan State University, East Lansing, MI 48824, U.S.A.

**Abstract**

*De novo* fatty acid biosynthesis occurs predominantly in plastids. The committed step for this pathway is the production of malonyl-CoA catalysed by acetyl-CoA carboxylase (ACCase). In most plants, plastidial ACCase is a multisubunit complex minimally comprised of four polypeptide chains, which catalyse two reactions. In the simple oilseed plant, *Arabidopsis thaliana*, two cDNAs encoding biotin carboxyl carrier protein (BCCP) isoforms have been identified. The remaining three subunits of ACCase appear to be single gene members in *A. thaliana* [Mekhedov, Martinez de Iarduy and Ohlrogge (2000) Plant Physiol. 122, 389–401]. Transcript and protein analyses indicate that BCCP isoform 1 is constitutively expressed while isoform 2 is predominantly expressed in developing seeds. The apparent masses of constitutive and seed-enriched BCCP isoforms agree with the apparent masses of recombinantly expressed isoforms 1 and 2.

---

Key words: acetyl-CoA carboxylase, fatty acid biosynthesis, oilseed. Abbreviations used: ACCase, acetyl-CoA carboxylase; BCCP, biotin carboxyl carrier protein.

*To whom correspondence should be addressed (e-mail thelen17@pilot.msu.edu).*

595 © 2000 Biochemical Society
respectively. In a related oilseed, *Brassica napus*, multiple putative BCCP polypeptides were also observed in developing seeds. The presence of a divergent class of BCCP genes in *A. thaliana* and *B. napus*, coincident with appropriately sized biotin-containing proteins expressed specifically in developing seeds, suggests that these BCCPs play an evolutionarily conserved role in oil deposition.

### Introduction

One important control point for fatty acid biosynthesis is the carboxylation of acetyl-CoA to form the three-carbon precursor malonyl-CoA (for review see [1]). This reaction is catalysed by a plastid-localized acetyl-CoA carboxylase (ACCase). In most plants, plastidial ACCase is a multisubunit complex comprised of at least four different polypeptides. The biotin carboxylase subunit catalyses the carboxylation (from bicarbonate) of biotin carboxyl carrier protein (BCCP). The subsequent carboxyl-transfer reaction is catalysed by a carboxyltransferase comprised of α and β subunits [2]. This two-step reaction is facilitated by BCCP, which must visit the active site of both biotin carboxylase and carboxyltransferase for each molecule of malonyl-CoA formed [3]. Gene redundancy for the BCCP subunit was reported previously from *Brassica napus* and *Glycine max* sources; the significance of this gene complexity is not yet known [4,5]. Herein we present evidence that a second, divergent class of BCCPs in Brassicaceae plants is highly expressed in developing seeds.

### Results and discussion

Evidence for multiple genes encoding BCCP subunits of the plastidial ACCase has been reported from *B. napus* and *G. max* sources [4,5]. Both of these plants are allotetraploids and this genetic complexity is generally reflected in higher gene numbers. In the simple genome of *Arabidopsis thaliana* two cDNAs encoding BCCPs that share approx. 30% amino acid identity have been identified [6,7]. Both contain plastid transit peptides and a conserved C-terminal biotinylation motif. Moreover, the recombinant proteins for both isoforms are biotinylated in *Escherichia coli* host cells [7,8] and J. J. Thelen, unpublished work). The second isoform was also shown to be competent for import into intact pea chloroplasts (J. J. Thelen, unpublished work). These data support the premise that both cDNAs encode BCCP subunits to the plastidial ACCase.

The dendrogram in Figure 1(A) illustrates the evolutionary relationship among plant BCCP polypeptides identified from expressed-sequence-tag databases at the time of writing. As a point of reference, the two most closely related polypeptides, *A. thaliana* 2 and *B. napus* 4, share 75% amino acid identity. All BCCP polypeptides represented in Figure 1(A) possess the hallmark C-terminal biotinylation motif QVXCIIEAMKL-MNEIE, harbouring the biotinyl-Lys residue [8]. Plant BCCPs are grouped into at least two evolutionary classes defined by the two *A. thaliana* isoforms. The genes for both *A. thaliana* isoforms are located on chromosome 5 and intron-exon length and position similarity (Figure 1B) suggests that the second isoform arose from an ancestral gene duplication. Since these isoforms sort into two divergent classes with *B. napus* BCCPs, we surmised that they might have distinct roles in fatty acid biosynthesis. To gain insight into what roles these might be, expression analyses were performed.

*A. thaliana* BCCP1 transcript was observed in roots, leaves, flowers and siliques and could thus be described as a constitutively expressed transcript (J. J. Thelen, unpublished work). In contrast, the transcript for isoform 2 was predominant in flowers and siliques and overall abundance was approx. 4-fold lower than of isoform 1 (J. J. Thelen, unpublished work). The deduced amino acid sequences of these two polypeptides exhibit only 30% amino acid identity, pointing to the possibility that they could be resolved by onedimensional SDS/PAGE. This was verified by recombinant expression of the mature polypeptides in *E. coli*. The apparent masses of recombinant isoforms 1 and 2 (fused to 42-amino acid N-terminal tags) were 42 and 32 kDa, respectively (Figure 1C). The 10-kDa difference in size agrees with the size difference between two biotin-containing proteins (35 and 25 kDa) found in developing *A. thaliana* seeds (Figure 1C). Although the apparent sizes of the native polypeptides are approx. 7 kDa less than the recombinant forms, this is probably due to 42-residue fusions at the N-termini. The 35-kDa biotin-containing protein is expressed in all *A. thaliana* organs while the 25-kDa form was observed only in developing seeds and flowers (Figure 1B, J. J. Thelen, unpublished work). Based upon apparent size and expression analyses, the 35- and 25-kDa
Figure 1
Clustal (A) and immunoblot (C) analyses and gene organization (B) of BCCPs from oilseed plants

(A) Alignment was performed using GeneWorks Software (Intelligenetics, Mountain View, CA, U.S.A.) with default parameters. Horizontal lines indicate inverse degree of relatedness. GenBank accession numbers are as follows: At 1, AF236873; At 2, AF223948; Bn 1, X90727; Bn 2, X90728; Bn 3, X90729; Bn 4, X90730; Bn 6, X90731; Bn 7, X90732; Gm, L 14863. Abbreviations: At, A. thaliana; Bn, B. napus; Gm, G. max. (B) Gene organization of A. thaliana BCCP isoforms. A scale model of intron and exon (boxes) organization for A. thaliana BCCP isoforms 1 and 2 is illustrated. GenBank accession numbers are as follows: At 1, AB005242; At 2, AP002074. (C) Immunoblot analyses of recombinant A. thaliana BCCPs and biotin-containing proteins from plant cell extracts probed with anti-biotin IgG-alkaline phosphatase-conjugated antibodies. Lanes 1 and 2 (reading from the left) are recombinant A. thaliana BCCP isoforms (0.5 μg each) expressed as fusion proteins with a 42-residue affinity tag. Lanes 3–6 are immunoblots of plant extracts (50 μg of protein) obtained by pulverizing fresh tissue directly in SDS/PAGE sample buffer [8 M urea/0.2% (w/v) SDS/2% (v/v) 2-mercaptoethanol]. Major biotin-containing polypeptides are indicated by arrows. Abbreviations: DAF, days after flowering; WAF, weeks after flowering.

Biocatalyst-containing polypeptides probably correspond to BCCP1 and 2, respectively. Analysis of biocatalyst-containing proteins in B. napus seeds showed multiple putative BCCPs in comparison with leaf tissue. These BCCPs are likely to correspond to the six isoforms identified previously from B. napus [4].

The presence of BCCP isoforms enriched in developing seed from Brassicaceae plants is possibly an adaptation for oil accumulation. The number of these seed-expressed isoforms in Brassicaceae plants varies from one (A. thaliana) to as many as four (B. napus), reflecting genome complexity. It is peculiar that the only multigenic subunit of A. thaliana plastid ACCase is BCCP, which has no catalytic activity. High expression of this isoform in developing seeds, where fatty acid biosynthesis is at a premium, points to the regulatory role of ACCase and more specifically to the biocatalyst-containing subunit of this complex. Perhaps BCCP expression is of regulatory importance for flux through ACCase. We are currently investigating this possibility through molecular-genetic approaches.

References
Effects of manipulating expression of acetyl-CoA carboxylase I in *Brassica napus* L. embryos

C. Sellwood*, A. R. Slabas† and S. Rawsthorne*

*Brassica and Oilseeds Research Department, John Innes Centre, Colney Lane, Norwich NR4 7UH, U.K., and †Department of Biological Sciences, University of Durham, Science Laboratories, South Road, Durham DH1 3LE, U.K.

Abstract

Acetyl-CoA carboxylase I (ACCase I) in developing oilseed rape embryos is predominantly cytosolic, based upon measurement of its propionyl-CoA carboxylase activity. Reduction of ACCase I by antisense expression reduces seed lipid content and affects carbohydrate metabolism.

Introduction

Acetyl-CoA carboxylase (ACCase) catalyses the first committed step in fatty acid biosynthesis [1]. There are two isoforms known in higher plants. ACCase I is a multifunctional homodimer capable of carboxylating acetyl-CoA or propionyl-CoA [i.e. it has a propionyl-CoA carboxylase (PCCase) activity]. This predominantly cytosolic isoform provides malonyl-CoA for, amongst other pathways, the elongation of fatty acids and synthesis of waxes [2,3]. ACCase II is a plastidial heteromer, consisting of four non-identical monomers, which carries out the acetyl-CoA carboxylation reaction only and in dicotyledonous plants is responsible for *de novo* fatty acid biosynthesis. *Brassica napus* L. is the only species reported to date that also has an isozyme of ACCase I present in the plastid as well as ACCase II [4,5]. We have now determined the distribution of the PCCase activity of ACCase I in developing embryos of wild type *B. napus* c.v. Topas.

*B. napus* c.v. Westar Double Haploid (Westar DH) plants expressing a partial antisense cDNA of cytosolic ACCase I in the embryo have a wrinkled phenotype and a dramatically reduced lipid content in the mature seed [6]. Analyses of storage-product accumulation (lipid, protein, soluble sugar and starch) in developing and mature embryos of the wild-type and two independent, homozygous transgenic lines (*B. napus* c.v. C4S6-16 and C7S1-2) have been carried out. The activity of PCCase has been determined in late-cotyledon-stage embryos from these three lines.

Experimental

*Brassica napus* L. plants were grown, siliques and embryos harvested, and total homogenates and plastid fractions prepared as described previously [7,8], except that embryos were homogenized in a total volume of 20 ml using razor blades. PCCase distribution was determined according to the principle described for measuring the distribution of cytosolic and plastid isoforms of glycolytic enzymes [9]. Carbon partitioning into the four key storage products, lipids [10], protein [11], starch [12] and soluble sugars [13], was measured in developing and mature seeds.

Results and discussion

Distribution of the PCCase activity of ACCase I

PCCase activity catalysed by ACCase I was used to monitor changes in the ACCase I distribution and amount in developing embryos of wild-type *B. napus* c.v. Topas. Total PCCase