Fatty Acid Biosynthesis: Elongation

Enzymic activities and gene expression of enzymes of the acyl-CoA elongase during rapeseed development

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Abstract

Enzymic activities and gene expression of oleoyl-CoA elongase were studied during seed development using two different rapeseed cultivars, high-erucic-acid rapeseed (HEAR) and low-erucic-acid rapeseed (LEAR). The overall elongase activities were maximal in HEAR between the fourth and eighth weeks after pollination (WAP) and absent in LEAR. The 3-ketoacyl-CoA synthase (condensing enzyme, CE) mRNA levels and the developmental profiles in the two cultivars were different since maximal expression levels were detected in HEAR and LEAR at WAP 4 and WAP 6, respectively. Anti-CE antibodies revealed two proteins of 60 and 67 kDa in both cultivars and an additional reacting protein of 57 kDa in HEAR.

Introduction

Erucic acid (C22:1), which is the product of the elongation of oleoyl-CoA by malonyl-CoA, is the major fatty acid of the oil from high-erucic-acid rapeseed (HEAR). Erucic acid biosynthesis is catalysed by the membrane-bound oleoyl-CoA elongase complex through four successive reactions: condensation (condensing enzyme, 3-ketoacyl-CoA synthase, CE), keto-acyl-CoA reduction (3-ketoacyl-CoA reductase), dehydration (3-OH-acyl-CoA dehydratase) and enoyl-CoA reduction (trans-2,3-enoyl-CoA reductase) [1]. The elongation mechanism is now well characterized and FAE1 genes (encoding CE) have been isolated [2] but the organization of the acyl-CoA elongase in the membrane, the developmental regulation of very-long-chain mono-unsaturated fatty acids (VLCMFA) and triacylglycerol biosynthesis remain unclear. Nevertheless, a thorough understanding of the physiological and genetic regulation of VLCMFA and triacylglycerol biosynthesis in seeds is fundamental to the rational development of biotechnological strategies for increasing seed oil content and manipulating oil composition.

The pioneering work by Norton and Harris [3] reported that the changes in fatty acid composition in developing rapeseed occur according to three consecutive phases and that erucic acid accumulation takes place in the last phase. Up to now, most of the studies have been carried out using microspore-derived cell cultures [4]. They have shown that the fatty acid synthesis and acyltransferase activities increase from the second to the fourth weeks of growth and then decrease [5,6]. The diacylglycerol acyltransferase activity and the oleosin gene expression increase when the sucrose concentration in the culture medium is raised from 2 to 22% (w/v). Abscisic acid and temperature stimulate erucic acid biosynthesis independently [7], leading to an accumulation of...
VLCMFA correlated with a stimulation of the elongase activity, which is 60% higher than in the untreated embryos [8]. The expression of CE transcripts is induced by 10 μM abscisic acid within 1 h and further increased up to 6 h while, at the same time, the VLCMFA content doubles [9].

In view of the lack of information concerning the regulation of erucic acid in *Brassica napus*, we investigated the enzymic activities and gene expression of the enzymes of the acyl-CoA elongase during rapeseed development by using two different cultivars, Gaspard (HEAR) and ISLR4 (low-erucic-acid rapeseed, LEAR), the latter being unable to produce C_{22:1}.

### Materials and methods

Garpard and ISLR4 seeds were field grown at INRA and collected during the spring of 1998 (Le Rheu, France). Microsomal membranes (100000×g pellet) and elongation activities (oleoyl-CoA and ATP-dependent elongases) were measured as already described [10]. RNA preparations were performed using a Qiagen RNeasy plant kit. The reverse transcriptase PCR (RT-PCR) of the mRNA encoding CE was carried out using a Qiagen RNeasy plant kit. The reverse transcriptase PCR (RT-PCR) of the mRNA encoding CE was carried out using 5'-ATCGTAGACGGTCCAAGTAC-3' and 5'-GACCCTAAAGCAATCTGCCA-3' as primers. RT-PCR was conducted with 40 cycles of 1 min at 94 °C, 45 s at 60 °C and 1 min at 72 °C followed by a final extension step of 10 min at 72 °C. Amplified cDNA was resolved by 2% agarose electrophoresis. The amplified cDNA was also used to synthesize a radiolabelled probe by random priming. Microsomal proteins were extracted, resolved by SDS/PAGE and immunodetection carried out using CE anti-serum and the ECL® kit (Amersham).

### Results and discussion

#### Elongase activities during seed development

The overall oleoyl-CoA and ATP-dependent elongase activities were measured as reported previously by Domergue et al. [10]. All measurements were carried out using microsomes prepared from seeds at weekly intervals from 1 to 9 weeks after pollination (WAP). Both activities were maximal in HEAR between the fourth and eighth WAP, while no activity was detected in LEAR (Table 1). Maximal activities were 18.6 and 14.3 nmol/mg per h for oleoyl-CoA and ATP-dependent elongases, respectively. Fatty acid analyses showed that C_{20:1}, C_{22:1} and C_{24:1} were synthesized at the same rate and that erucic acid was the major product.

### CE gene expression during seed development

The pattern of CE mRNA was studied using Northern hybridizations. The 1.6-kb CE mRNA was expressed in both cultivars at similar levels. However, the developmental profiles were different since maximal expression levels were detected in HEAR and LEAR at WAP 4 and WAP 6, respectively. Two homologues of the *FAE1* gene, CE7 and CE8, have been identified so far in the

<table>
<thead>
<tr>
<th>Seed development</th>
<th>3 WAP</th>
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<th>5 WAP</th>
<th>6 WAP</th>
<th>8 WAP</th>
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<tr>
<td>Oleoyl-CoA elongase (nmol/mg per h)</td>
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<td>HEAR</td>
<td>2.4</td>
<td>7.9</td>
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<td>ATP-dependent elongase (nmol/mg per h)</td>
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<td>HEAR</td>
<td>0.7</td>
<td>9.0</td>
<td>14.3</td>
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<td>CE mRNA expression (arbitrary units)</td>
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<tr>
<td>HEAR</td>
<td>15</td>
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<td>44</td>
<td>33</td>
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<tr>
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<td>46</td>
<td>43</td>
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<td>Immunodetection 57-kDa protein</td>
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rapeseed genome. Taking advantage of the presence of a HindIII restriction site only present in the CE8 coding region, an RT-PCR assay coupled with a digestion step was used to measure the relative expression of the CE7 and CE8 mRNAs. In both cultivars, CE7 mRNA was the predominant isoform expressed throughout the developmental period of the seed studied.

Immunodetection of CE during seed development

Western blots were performed using anti-CE antibodies prepared against a CE recombinant protein [11]. In both cultivars, the immunoblots revealed two proteins of 60 and 67 kDa from the earliest stages of seed development. These proteins may correspond to ubiquitous condensing-enzyme proteins involved in fatty acid production [11]. However, an additional 57-kDa protein was detected only in the 5-8 WAP HEAR samples.

In conclusion, our results suggest that: (i) the CE gene codes for a native 57-kDa protein; (ii) this protein occurs only in the Gaspard cultivar; (iii) this protein is probably the active CE enzyme and (iv) the mutations leading to the LEAR phenotype may induce a disregulation of the expression of the CE gene at the translational or post-translational levels.

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References


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Biochemical and molecular characterization of corn (Zea mays L.) root elongases

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Abstract

Root surfaces are protected against the soil environment by the deposition of lignin and suberin. In order to obtain more insight into the regulation of root suberin biosynthesis, elongases from primary roots of corn (Zea mays L.) seedlings were characterized. Elongase activities (acyl-CoA and ATP-dependent) were located in the microsomal fraction of the root cells. C₁₈, C₂₀ and C₂₂ fatty acids were detected as primary products of elongases. Preferred substrates of the acyl-CoA elongases were C₁₈, C₂₀-CoA and C₂₀, C₂₂-CoA. Applying a molecular approach, using PCR and degenerate primers derived from the sequences of 3-ketoacyl-CoA synthases (KCSs), catalysing the first step of very-long-chain fatty acid synthesis, the cDNA of a putative root KCS was obtained showing high homology to known leaf and seed KCSs at the DNA and amino acid levels. Thus, our approach provides the first direct evidence for the presence and the activity of root elongases in Z. mays. Ongoing research is focusing on the molecular analysis and the regulation of KCS expression in roots in reaction to different environmental stimuli.

Key words: 3-ketoacyl-CoA synthase, suberin, very-long-chain fatty acid.
Abbreviation used: VLCFA, very-long-chain fatty acid.
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