The Arabidopsis acyl-CoA oxidase gene family
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Abstract
A family of acyl-CoA oxidase isozymes catalyse the first step in the peroxisomal fatty acid β-oxidation spiral. Our group and others have recently characterized four genes from this family in the model oilseed Arabidopsis. These genes encode isozymes with different acyl-CoA substrate specificities, which together encompass the full range of fatty acid chain lengths that exist in vivo. Here we review the biochemical properties and physiological roles of the acyl-CoA oxidase isozymes.

Introduction
Peroxisomal β-oxidation is the major pathway of fatty acid catabolism in higher plants. This pathway plays various important physiological roles. These include storage lipid reserve mobilization during oilseed germination [1], senescence [1] and starvation [2], membrane-lipid turnover and the synthesis of fatty acid-derived signals (e.g. jasmonic acid) [3].

The first step in β-oxidation is catalysed by acyl-CoA oxidase [1] (ACX, EC 1.3.3.6). This enzyme catalyses the conversion of fatty acyl-CoAs to trans-2-enoyl-CoAs and is believed to be important in exerting control over the rate of carbon flux through the pathway [4,5]. The reaction requires FAD as a cofactor. FAD is subsequently re-oxidized by O2 to form H2O2, which is converted to H2O by catalase.

Biochemical evidence suggested that plants contain a family of ACX isozymes with distinct but partially overlapping acyl-CoA chain-length specificities [6–8]. Plant cDNAs with homology to animal and fungal ACXs have previously been reported [9–11]. However, until recently genes encoding these enzymes remained to be characterized functionally.

Identification of Arabidopsis ACX isogenes
Four ACX isogenes have now been isolated from the model oilseed Arabidopsis [12–14]. Work from our group lead to the cloning and characterization of AtACX1 and AtACX2 [12]. These genes were initially identified in the Arabidopsis expressed sequence tag (EST) database as partial cDNA sequences with homology to yeast and human ACX genes [12]. Using the same EST database Hayashi and co-workers [13] identified a gene with homology to mammalian mitochondrial acyl-CoA dehydrogenases (ACADs). Sequencing of the cDNA revealed that the derived amino acid sequence contained a typical peroxisomal targeting signal (PTS1) at the C-terminus. Since ACX and ACAD are structurally and functionally related they suggested that the clone might encode an ACX (referred to here as AtACX4). Finally a fourth ACX from Arabidopsis (AtACX3) has recently been isolated [14,15]. Froman and co-workers [14] identified a partial genomic clone of this gene via homology, whereas in our laboratory AtACX3 was identified following the isolation of a promoter-trapped mutant which displayed expression of the reporter gene (gusA) in the root meristem [15]. ACX is known to be highly active in the root tip [2].

The four isoenzymes share significant levels of homology at the amino acid level. Furthermore, all contain tightly conserved regions homologous to mammalian ACAD protein signatures 1 and 2 [16]. It has been suggested that these regions may be important for interaction with the substrates (e.g. acyl-CoA) [13]. AtACX1, AtACX2, AtACX3 and AtACX4 are located on chromosomes IV, V, I and III, respectively [14]. Available evidence suggests that AtACX2, AtACX3 and AtACX4 are single-copy genes while an AtACX1 homologue has recently been reported [14].

Biochemical properties
The biochemical properties of all four ACX genes have been studied [12–15] (Table 1). Full-length cDNA clones of AtACX1 and AtACX2 were overexpressed in Escherichia coli [12]. Analysis of
Table 1

Properties of Arabidopsis ACX isoforms

Sources of information: ACX1 and ACX2 [12], ACX3 [15] and ACX4 [13]. GenBank accession numbers for the corresponding cDNAs (ACX1–4) are AF057043, AF057044, AF253474 and AB017643, respectively.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Substrate</th>
<th>(K_m) ((\mu)M)</th>
<th>pH optimum</th>
<th>pl</th>
<th>Molecular mass (kDa)</th>
<th>Native state</th>
<th>Targeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACX1</td>
<td>14:0-CoA</td>
<td>5.3</td>
<td>8.5</td>
<td>7.6</td>
<td>73</td>
<td>Dimer</td>
<td>PTS1</td>
</tr>
<tr>
<td>ACX2</td>
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<td>8.5</td>
<td>8.2</td>
<td>77</td>
<td>Dimer</td>
<td>PTS2</td>
</tr>
<tr>
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<td>3.7</td>
<td>8.5</td>
<td>8.2</td>
<td>76</td>
<td>Dimer</td>
<td>PTS2</td>
</tr>
<tr>
<td>ACX4</td>
<td>6:0-CoA</td>
<td>8.3</td>
<td>8.5–9.0</td>
<td>9.5</td>
<td>47</td>
<td>Tetramer</td>
<td>PTS1</td>
</tr>
</tbody>
</table>

substrate specificities of the recombinant proteins revealed that AtACX1 encodes an enzyme with affinity for medium-to-long-chain saturated acyl-CoAs while AtACX2 encodes an enzyme with affinity for long-chain unsaturated acyl-CoAs. The AtACX2 amino acid sequence was similar to a pumpkin cDNA previously designated as a long-chain acyl-CoA oxidase based on immunological evidence [11]. Hayashi and co-workers [13] overexpressed the ACAD homologue (AtACX4) in a baculovirus expression system and showed that the protein has short-chain ACX activity. Seedlings of the acx3 mutant show a deficiency in medium-chain ACX activity while long- and short-chain activities are unchanged [15]. Overexpression of the ACX3 protein in E. coli confirmed that ACX3 has medium-chain ACX substrate specificity (Table 1). The Arabidopsis ACX gene family therefore comprises four genes with substrate specificities that encompass the full range of acyl-CoA chain lengths that exist in vivo.

The long-, medium-to-long- and medium-chain ACX isozymes share common characteristics in terms of their affinity for their respective substrates, subunit molecular masses and native aggregation states (Table 1). However, short-chain ACX differs in that it has approximately double the \(K_m\) of the other isozymes and is a homotetramer rather than a dimer (Table 1). Phylogenetic analysis shows that ACX4 clusters with ACADs rather than ACXs [13]. Interestingly, mammalian peroxisomes lack the ability to \(\beta\)-oxidize short-chain acyl-CoAs and ACX4 is therefore unique to plants [13]. It has been suggested that ACX4 might have arisen from mitochondrial ACADs, gaining a PTS during evolution [13].

All the members of the ACX gene family are likely to encode peroxisomal proteins. The localization of ACX4 and ACX3 have been demonstrated by cell-fractionation experiments and/or immunolocalization [13,15]. Both ACX1 and ACX4 have C-terminal PTS1 targeting signals [12,13]. ACX2 and ACX3 contain a putative PTS2 at the N-terminus [12,14]. The pH optima of all four isozymes are similar and in the alkaline range, which agrees with a recent report that the peroxisome matrix is basic [17].

Gene expression and enzyme activity

Transcript and/or protein levels for all four genes increase dramatically during Arabidopsis seed germination [12,13,15]. Levels peak approx. 2 days after seed imbibition when lipid reserves are being mobilized most rapidly and thereafter decline. We have used the promoter-trapped acx3 mutant line to demonstrate that AtACX3 is regulated predominantly at the level of transcription during germination [15]. The transcript abundance of AtACX1 and AtACX2 is 3–5-fold greater in 2-day-old seedlings than in 7-day-old plants [12]. These differences are reflected closely by ACX activities (Table 2).

Table 2

ACX activities in Arabidopsis seedlings

Seedlings (ecotype Col0) were grown and activities measured as described in [12] using various acyl-CoAs as substrate. Values are the means±SEM of measurements made on three separate batches of seedlings.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>2 days old</th>
<th>7 days old</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:1-CoA</td>
<td>3.9±0.4</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>14:0-CoA</td>
<td>11.2±1.4</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>12:0-CoA</td>
<td>32.4±7.1</td>
<td>8.1±1.9</td>
</tr>
<tr>
<td>6:0-CoA</td>
<td>45.3±8.4</td>
<td>17.1±2.3</td>
</tr>
</tbody>
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Lipid Catabolism: Fatty Acid Breakdown

The activities of short-chain and medium-chain ACXs are around 10-fold higher than the long-chain activity (Table 2). This pattern is consistent at the two stages of seedling development shown in Table 2 and is also found in mature leaves of *Arabidopsis* [18]. Seedlings of *acx3* contain less than 5% of wild-type medium-chain ACX activity yet lipid catabolism is not impaired during germination [15]. These data are consistent with the hypothesis that among the ACX isozymes long-chain ACX plays the predominant role in controlling the rate of fatty acid breakdown.

Conclusions

In conclusion our group and others have cloned and characterized *ACX* isogenes from *Arabidopsis*. This family is composed of at least four isozymes with different substrate chain-length specificities. Together they can account for the catabolism of the full range of fatty acids present in *oioa*. Further work will be required to determine the physiological role(s) of these genes, both as a family and individually.

References


Contribution of mitochondria and peroxisomes to palmitate oxidation in pea tissues

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Abstract

Total, mitochondrial and peroxisomal palmitate oxidation capacities were compared in pea, from the dry seed to 14 days after imbibition. Total β-oxidation varied over the measured time period and showed four peaks of activity at day 2, days 5-6, day 10 and days 12-13. The contribution of peroxisomal and mitochondrial β-oxidation to this overall β-oxidation varied. Over the first 48 h of seed germination, peroxisomal β-oxidation accounted for 80-100% of the total observed β-oxidation. The larger peaks of β-oxidation at days 5-6, day 10 and days 12-13 were due primarily to mitochondrial β-oxidation activity, which accounted for 70-90% of the observed total β-oxidation at these times. The peaks of activity are related to observed stages in seedling development.

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