Abstract

We have identified two families of acyl-CoA thioesterase (ACHs) in Arabidopsis thaliana. One family, consisting of AtACH1 and AtACH2, appears to be peroxisomal, as they have type-1 peroxisomal targeting sequences. The other family, consisting of AtACH4 and AtACH5, resides in the endoplasmic reticulum, as shown by green fluorescent protein studies. AtACH2 has been overexpressed in Escherichia coli and shows high levels of acyl-CoA thioesterase activity against both 16:0-CoA and 18:1-CoA. AtACH5 has also been overexpressed in E. coli, and shows thioesterase activity as well. ACHs have been characterized in other many other organisms and in various subcellular locations, but their true physiological role is not yet understood. Indeed, atach5 gene knockout mutants have no observable phenotype.

As the oilseed technology industry seeks to create special plants with 'designer oils', a better understanding of the enzymes which affect the basic units of lipid synthesis will be important. One group of enzymes which may regulate the characteristics of the plant lipids is the acyl-CoA thioesterases (ACHs). These enzymes hydrolyse fatty acyl-CoAs, basic units of lipid synthesis, yielding free fatty acid and CoA (see Figure 1).

Removing the CoA group from the fatty acid prevents its incorporation into lipids. Since these enzymes may be responsible for watching over and eliminating 'irregular' acyl-CoAs from the acyl-CoA pool, removing their activity would be important in plants that have been engineered to produce high levels of lipid containing irregular fatty acids. ACHs have been cloned and characterized in organisms from Escherichia coli [1] to humans [2], and are localized to different areas of the cell, such as the peroxisome [3] or the mitochondrion [4]. However, despite efforts to define what role these enzymes play in the different organelles, their true physiological function is still unknown.

We were able to identify sequences in the Arabidopsis thaliana database that are homologous to other known ACHs. One group, made up of AtACH1 and AtACH2, carries the type-1 peroxisomal targeting sequences. These enzymes are homologous to E. coli thioesterase II (TESB), whose three-dimensional structure was recently solved [5]. AtACH1 and AtACH2 have conserved amino acid residues at the proposed active site. AtACH2, which has been overexpressed in E. coli and partially purified, demonstrates strong ACH activity (see Figure 2). Exactly what part these enzymes play in the peroxisome is still not understood, although yeast cells with missing ACH activity in the peroxisome grow poorly in oleate media [3], suggesting that these enzymes are involved in β-oxidation.

The second family of ACHs, consisting of AtACH4 and AtACH5, reside in the endoplasmic...

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Figure 1
ACH activity

[Diagram of ACH activity]

Key words: lipid synthesis, peroxisome.
Abbreviation used: ACH, acyl-CoA thioesterase.
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Abstract
Seed-specific expression in Arabidopsis thaliana of oleate hydroxylase enzymes from castor bean and Lesquerella fendleri resulted in the accumulation of hydroxy fatty acids in the seed oil. By using various Arabidopsis mutant lines it was shown that the endoplasmic reticulum (ER) n-3 desaturase (FAD3) and the FAE1 condensing enzyme are involved in the synthesis of polyunsaturated and very-long-chain hydroxy fatty acids, respectively. In Arabidopsis plants with an active ER Δ12-oleate desaturase the presence of hydroxy fatty acids corresponded to an increase in the levels of 18:1 and a decrease in 18:2 levels. Expression in yeast indicates that the castor hydroxylase also has a low level of desaturase activity.

References

Received 7 August 2000

Production of hydroxy fatty acids in the seeds of Arabidopsis thaliana
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Introduction
Hydroxy fatty acids accumulate in the seeds of a small number of plant species. Of these, castor bean (Ricinus communis) is the most well known, producing a seed oil containing 85–90 %, ricinoleic acid (18:1-OH). This fatty acid is a valuable raw material and is used in a variety of applications ranging from the manufacture of Nylon and speciality lubricants to paints and cosmetics. Castor oil is the only significant commercial source of hydroxy fatty acids but is less than ideal as the seeds contain potent toxins and allergens. There is considerable interest in finding an alternative supply of hydroxy fatty acids, either from another species of plant (such as members of the Lesquerella genus), or by the genetic engineering of an existing crop species.

In castor, 18:1-OH is formed by the direct Δ12-hydroxylation of oleic acid (18:1) esterified to position sn-2 of phosphatidylcholine [1]. The reaction is catalysed by a protein almost identical to an endoplasmic reticulum (ER) Δ12-desaturase. A cDNA encoding the castor Δ12-oleate hydroxylase has been isolated [2,3] and a similar sequence,