Acyl-CoA dehydrogenase activity in pea cotyledon tissue during germination and initial growth

C. Masterson, A. Blackburn and C. Wood
Department of Biological and Nutritional Sciences, Agriculture Building, Kings Road, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.

Abstract
Acyl-CoA dehydrogenase activity has been measured in homogenates of post-imbibition to 14-day-old hydroponically grown pea seeds at daily intervals, using C₁₄, C₁₆ and C₁₈ acyl-CoA substrates. The activity peaks of the different chain-length acyl-CoA dehydrogenases did not transpose at all points and the ratios of the chain-length activities were not constant. It therefore has to be concluded that more than one dehydrogenase is present in pea mitochondria. There was a post-imbibition initial surge of activity with short- and mid-chain-length substrates. The C₁₆-handling enzyme first peaked at 3–4 days, which coincided with the onset of plumule unfurling and greening. Further peaks were observed with all three substrates, coinciding with secondary root formation and leaf enlargement and later with cotyledon degeneration. Overall activity showed that the long-chain acyl-CoA dehydrogenase was much more active than the short-chain acyl-CoA dehydrogenase.

Introduction
The subcellular localization of β-oxidation of fatty acids in higher plants has been the subject of great debate over the last 15 years. The major consensus that β-oxidation is exclusively peroxisomal [1] has been challenged over recent years as increasing evidence in support of an additional, mitochondrial site has accrued, as reviewed by Masterson and Wood [2].

One of the characteristics which differentiates mitochondrial β-oxidation from peroxisomal β-oxidation in higher plants is the first enzyme of the four-enzyme β-oxidation spiral. In peroxisomes this enzyme is acyl-CoA oxidase (EC 1.3.3.6), an FAD-containing enzyme that is re-oxidized by direct transfer of electrons from FADH₂ to O₂ to form H₂O₂ with subsequent breakdown by catalase. Mitochondria possess acyl-CoA dehydrogenase (EC 1.3.99.2), also an FAD-containing enzyme, the FADH in this case being re-oxidized by the electron-transport chain.

Acyl-CoA dehydrogenase has been detected in purified mitochondria from maize roots [3,4], pea cotyledons [5] and sunflower seeds [4]. Activity from pea cotyledon mitochondria was detected with a wide range of acyl-CoA substrates [5], with peaks at C₁₄, C₁₆ and C₁₈ chain lengths indicating not one but a family of acyl-CoA dehydrogenases. Bode et al. [4] partially purified two proteins from sunflower seeds which exhibited acyl-CoA dehydrogenase activity, one favouring short-chain acyl-CoA substrates and the other mid-to-long-chain substrates. They also concluded that there is a family of enzymes within the mitochondria.

This current work further investigates the presence of an acyl-CoA dehydrogenase family of enzymes in pea cotyledons by measuring enzyme activity with substrates of different chain lengths in tissues at different developmental stages.

Materials and methods
Pea seeds (Pisum sativum L. cv Bunting) were surface sterilized in 6% (v/v) sodium hypochlorite for 5 min, imbibed in running tap water for 8 h and germinated on damp blotting paper at 25 °C for a further 40 h. The developing seedlings were grown hydroponically by suspension over trays of water for up to a further 12 days at room
temperature, with frequent water changes. Dry, unimbibed seeds and cotyledons from 1- to 14-day-old seedlings were used for enzyme preparations.

Crude organelle suspensions were prepared from cotyledons by a modified method of McNeil and Thomas [6]. Here, after a slow centrifugation to remove starch and cellular debris, the organelles were collected at 10000 \( g \) for 10 min. The pellet was resuspended in 7–10 ml of 10 mM Tris/HCl at pH 7.2 and immediately frozen in liquid nitrogen followed by thawing. This freeze-thawing ruptured the organelle membranes, which were removed by centrifugation at 144000 \( g \) for 30 min. The resulting soluble protein was precipitated with 100 \( \% (w/v) \) \((NH_4)_2SO_4\) over 30 min, collected by centrifugation at 144000 \( g \) for 30 min and resuspended in 1.5 ml of 10 mM Tris/HCl, pH 7.2, prior to enzyme assay.

Acyl-CoA dehydrogenase activity was measured by the method of Yao and Schultz [7] using butyryl-, lauryl- or palmitoyl-CoAs as substrate. Measured absorbance changes in the absence of substrate were subtracted from the rates in the presence of substrate to account for non-enzymic reduction of phenazine methosulphate and/or 2,6-dichlorophenolindophenol. Protein was measured by the method of Bradford [8] using BSA as a standard.

**Results and discussion**

As expected there was no acyl-CoA dehydrogenase activity in tissue from unimbibed seeds. Four main peaks of acyl-CoA dehydrogenase activity were observed at day 1, days 3–4, days 7–8 and day 11 after the commencement of imbibition (Figure 1).

Day 1 represents seeds that were fully imbibed and germinated with radicles apparent and approx. 2 mm long. There was a pronounced surge of activity with the short- and mid-chain-length substrates at this stage but no activity observed with the long-chain palmitoyl-CoA substrate.

Days 3–4 represent the emergence stage, with the main radicle about 70 mm long and the hooked plumule unfurling and greening. Days 7–8 represent development of the lateral radicles and enlargement of the still tightly folded leaves. Acyl-CoA dehydrogenase activity was observed with all three substrates at both of these peaks, although

![Figure 1](image-url)
the magnitude of the activities were differential with respect to chain length. In both cases the long-chain acyl-CoA dehydrogenase activity was greater than the activity with short- and mid-chain substrates.

Day 11 represents expansion and flattening of the leaflets of the first node and establishment of independent seedlings as the cotyledons begin to degenerate. There was a large surge of short- and long-chain acyl-CoA dehydrogenase activity at this stage.

As the peaks of the different chain-length acyl-CoA dehydrogenases do not transpose at all points and the ratios of the chain-length activities are not constant, it has to be concluded that more than one dehydrogenase is present in pea mitochondria, which is in accordance with previous reports [4,5]. These peaks of acyl-CoA dehydrogenase activity follow the same pattern as reported for overall mitochondrial \( \beta \)-oxidation [9]. \( \beta \)-Oxidation of fatty acids stored in the cotyledons will provide respiratory substrates for the generation of ATP to help fuel the initial growth of the seedling. \( \beta \)-Oxidation will also provide acetyl-CoA for biosynthesis. Additionally controlled partial \( \beta \)-oxidation in mitochondria has been implicated in fatty acid shortening for the biosynthesis of mid-chain fatty acids [10]. Both acetyl-CoA and mid-chain fatty acids may be used for synthetic purposes as the cotyledon store is utilized to supply metabolites for chloroplast-membrane biosynthesis during plumule and leaf greening and membrane biosynthesis during lateral root development. During cotyledon degeneration, fatty acids released by membrane breakdown may be oxidized and utilized by the growing seedling. Clearly much more work is needed in this area and the enzyme(s) require purifying and characterizing before speculation is replaced by fact.

We thank Batchelor Foods UK for their gift of pea seeds. C.M. thanks the Royal Society for her University Research Fellowship.

References

Received 15 June 2000

Arabidopsis thaliana mutants disrupted in lipid mobilization
P. R. Lange and I. Graham
Centre for Novel Agricultural Products, Department of Biology, University of York, CSL, Sand Hutton, York YO41 1LZ, U.K.

Abstract
To isolate mutants in the process of lipid mobilization during post-germinative growth we employed a screen using the pro-herbicide 2,4-dichlorophenoxybutyric acid (2,4-DB). The phenotypes of a number of 2,4-DB-resistant mutants are compared with previously characterized mutants disrupted in \( \beta \)-oxidation or the glyoxylate cycle. We conclude that the strength of 2,4-DB resistance and the ability of the seedlings to grow in the absence of exogenous sugar are inversely correlated. Sugar dependence of 2,4-DB-resistant seedlings is a consequence of impaired storage-lipid mobilization.

Introduction
In oilseeds such as Arabidopsis thaliana the catabolism of fatty acids supports germination until the seedling becomes photoauxotropic. The