The involvement of phospholipid: diacylglycerol acyltransferases in triacylglycerol production

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Abstract

We have characterized three CoA-independent types of enzyme, phospholipases, phospholipid: diacylglycerol acyltransferases (PDATs) and cholinephosphotransferases, responsible for the removal of unusual fatty acids from phosphatidylcholine (PC) in microsomal preparations from developing oil seeds. The metabolism of sn-2-[14C]acyl-PC was monitored in microsomal preparations from various oilseeds having either medium-chain, acetylenic, epoxy or hydroxy fatty acids as their major fatty acids in the oil. The results indicate that PDAT plays a major role in removing ricinoleic acid and vernolic acid from phospholipids in Ricinus communis and Crepis palaestina seeds, respectively. However, vernolic, crepenynic and capric acids are primarily removed from phospholipids by phospholipases in Euphorbia lagascae, Crepis rubra and elm seeds, respectively. Further, we show that significant PDAT activity is also present in vegetative tissues of Arabidopsis thaliana.

Introduction

Until recently, it was believed that only one enzyme, the diacylglycerol acyltransferase (DAGAT), could carry out the final step in triacylglycerol biosynthesis. We have, however, demonstrated that microsomal preparation of developing seeds from several plants as well as microsomal preparations from yeast catalyse triacylglycerol formation via a phospholipid: diacylglycerol acyltransferase (PDAT). This enzyme is structurally related to the lecithin: cholesterol acyltransferase [1] and appears to play a major role in removing unusual fatty acids from the phospholipids in some oilseeds. We show in this work that the relative contribution of PDAT in the removal of unusual fatty acids from phospholipids varies dramatically between oilseeds accumulating different unusual fatty acids and even between species accumulating the same type of unusual fatty acid to approximately the same level in the oil. Moreover, we show that PDAT activity is present in the vegetative parts of the plant. The physiological role of PDAT in these tissues, as well as in yeast, is not yet understood.

Materials and methods

[1-14C]Acyl groups of crepenynic, vernolic and ricinoleic acids were synthesized enzymatically by incubation of radioactive precursor fatty acids with microsomal preparations from Crepis alpina, Crepis palaestina and castor bean, respectively, according to [2]. sn-2-[14C]oleyl-phosphatidylcholine (PC) was synthesized chemically according to [3] whereas sn-2-[14C]acyl-PC with uncommon radioactive fatty acids was synthesized enzymatically [4]. Microsomes from the different tissues were prepared according to [5]. Incubation of microsomal preparations with radioactive PC and analysis of radioactive products were performed according to [1].

Results and discussion

The metabolism of sn-2-[14C]acyl-PC into free fatty acids, diacylglycerols (DAGs) and triacylglycerol, by the action of phospholipases, cholinephosphotransferase (CPT) and PDAT respectively, was studied in microsomal preparations from various developing oilseeds accumulating different types of unusual fatty acids. There was a large difference in the relative activity between the oilseeds in the metabolism of PC with unusual fatty acids (Figure 1). In all microsomal preparations there was a huge difference in the relative activity of the phospholipases and/or PDAT regarding the removal of unusual fatty acids from the PC substrate, as compared to oleic acids (Figure 1). Whereas PDAT in microsomes from elm seeds (accumulating capric and caprylic acid in the oil) and Crepis rubra seeds (accumulating crepenynic acid in the oil) was virtually

Key words: cholinephosphotransferase, lipid, phospholipases, phospholipid: diacylglycerol acyl transferase, triacylglycerol.

Abbreviations used: PDAT, phospholipid: diacylglycerol acyltransferase; PC, phosphatidylcholine; DAG, diacylglycerol; CPT, cholinephosphotransferase.

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inactive towards capryol-PC and crepenynoyl-PC, respectively, these fatty acids were efficiently and specifically removed by phospholipases in these microsomes. Phospholipase activity was also the dominating activity in the removal of vernolic acid in microsomes from *Euphorbia lagascae* seeds (accumulating vernolic acid in the oil), although some PDAT activity with specificity for vernoloyl-PC was also present. Microsomal preparations from *Ricinus communis* seeds (accumulating ricinoleic acids in the oil) and *C. palaestina* seeds (accumulating vernolic acid) removed ricinoleic and vernolic acids, respectively, highly efficiently and specifically via the action of PDAT but they also had some fatty acid-specific phospholipase activities. It is noteworthy that, in spite of the close evolutionary relationship between *C. palaestina* and *C. rubra* species as well as between the epoxygenase and acetylenase proteins [6], it appears that the two plant species have selected different strategies to remove their respective unusual fatty acids from PC. Also the two species that both accumulate vernolic acid, *E. lagascae* and *C. palaestina*, have selected two different strategies to remove this acid from the phospholipids.

It should be noted that the reversal action of CPT plays a significant role in transferring fatty acids from phospholipids into DAG in the two Crepis species and in *R. communis*. The great differences between the amount of DAG with unusual fatty acids as compared to oleoyl groups produced from PC in *C. palaestina* and *R. communis* microsomes (Figure 1) is not likely to be due to any oleoyl-specificity exerted by CPT per se, but to a rapid utilization of DAG with unusual fatty acids by PDAT in these species. However, the higher amount of vernoyl-DAG compared to oleoyl-DAG formed in *E. lagascae* microsomes suggests that the CPT, in certain species, actually can possess acyl specificity.

An orthologue to the yeast PDAT gene, derived from pooled mRNA of different tissues from *Arabidopsis thaliana*, was identified in the GenBank database (accession no. T04806). Therefore it was of interest to see whether we could demonstrate PDAT activity in vegetative tissue of this plant. Microsomes from shoots and roots of *A. thaliana* both showed significant PDAT activity with ricinoleoyl-PC and oleoyl-PC, although the relative activity between the two substrates did not differ as dramatically as in microsomes of *R. communis* (results not shown). It is plausible that the PDAT enzyme in yeast and vegetative tissues of plants plays another, quite different and as-yet-unidentified physiological role in oilseeds accumulating unusual fatty acids.

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**Figure 1**

Metabolism of sn-2-[14C]acyl-PC into free fatty acids (FFAs), DAGs and triacylglycerols (TAGs) in microsomal preparations from various developing oilseeds

The amount of radioactivity is calculated relative to the amount produced in the product with the highest amount of radioactivity in each plant species. Abbreviations: 10:0-PC, caproyl-PC; 18:1-PC, oleoyl-PC; crep-PC, crepenynoyl-PC; verm-PC, vernoloyl-PC; rnc-PC, ricinoleoyl-PC.
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References


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Accumulation of storage products in oat during kernel development

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Abstract

Lipids, proteins and starch are the main storage products in oat seeds. As a first step in elucidating the regulatory mechanisms behind the deposition of these compounds, two different oat varieties, ‘Freja’ and ‘Matilda’, were analysed during kernel development. In both cultivars, the majority of the lipids accumulated at very early stage of development but Matilda accumulated about twice the amount of lipids compared to Freja. Accumulation of proteins and starch started also in the early stage of kernel development but, in contrast to lipids, continued over a considerably longer period. The high-oil variety Matilda also accumulated higher amounts of proteins than Freja. The starch content in Freja kernels was higher than in Matilda kernels and the difference was most pronounced during the early stage of development when oil synthesis was most active. Oleosin accumulation continued during the whole period of kernel development.

Introduction

Among the cereals, only oat and maize have potential to be used as oil-producing crops. The oil content in the kernels of different oat cultivars varies from 3 to 12% of the dry weight, but breeding lines with even higher oil content (up to 19%) have been reported. Apart from oil, protein and starch are the main storage components of oats and these products vary also in the different cultivars. Protein levels ranging from 16 to 20% of dry weight and levels of starch from about 45 to 60% have been reported. The differences in the proportions of the different storage materials are not due to the morphological differences in oat kernels between the different cultivars. Therefore, it is likely that the observed differences are due to differences in intracellular enzyme activities in the different kernel tissues. As a first step to elucidating the regulatory mechanisms behind different storage product’s deposition in oat, we present here data concerning accumulation of lipids, proteins and starch in medium-oil and high-oil oat varieties during kernel development.

Materials and methods

The oat cultivars ‘Freja’ and ‘Matilda’ (Svalöf Weibull AB, Svalöf, Sweden) were grown in a greenhouse with a supplement of artificial light. Seeds at three different stages of development as well as mature seeds were used for analysis. Stage 1: seeds were about 2 mm long with a fresh weight of about 7 mg. Stage 2: seeds were still green, with a milky endosperm and a fresh weight of about 40–45 mg. Stage 3: seeds were pale green with a jelly-like endosperm and fresh weight of about 55–60 mg. Stage 4: mature seeds with a fresh weight of about 40 mg. Lipid content were determined by extraction of the lipids from the kernel [1] and determination of the amount of fatty acid methyl esters was by GLC after transmethylation. Soluble proteins were extracted from freshly harvested oat seeds. This was achieved by homogenization with an Ultra-Turrax® followed by extraction with 0.1 M Tris/HCl buffer (pH 6.8)