ALTERATION OF THE FATTY ACID COMPOSITION OF RAPE SEED OIL BY BIOCHEMICAL AND MOLECULAR BIOLOGICAL APPROACHES

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INTRODUCTION

The technical use of rapeseed oil could be expanded by a more uniform esterification of all three positions of the glycerol backbone with one species of fatty acids. In this context the production of rapeseed oil with a high proportion of very long chain fatty acids (>C18) is of major interest. Up to now breeding has led to varieties of rapeseed (Brassica napus) containing more than 50% erucic acid (C22:1) in their triacylglycerols (TAG) but never exceeded 66%. Here we report on approaches and results to pinpoint the reason for this limit and the strategy to overcome this block.

RESULTS & DISCUSSION

Position analyses of TAG from high erucic rapeseed varieties revealed that C22:1 is found only in position 1 and 3 of the glycerol backbone, but not in position 2 (Norton and Harris 1986).

The absence of C22:1 at position 2 is due to the properties of the acyl-CoA:1-acylglycerol-3-phosphate acyltransferase (LPA-AT)(EC 2.3.1.51). This enzyme, which catalyzes the acylation reaction at position 2, does not accept C22:1-CoA as a substrate in ripening rapeseeds (Bernerth and Frentzen 1990). Our strategy to overcome this block is the following:

- Identification of a LPA-AT which utilizies C22:1-CoA.
- Isolation of the corresponding gene.
- Modification of the gene to ensure proper expression in developing seeds.
- Introduction of this modified gene into rapeseed plants.

Plants with a high content of very long chain fatty acids in the TAG were screened for the substrate specificities of their LPA-AT. In ripening seeds of Limnanthes douglasii an enzyme was found with the property to incorporate C22:1 in position 2 of TAG (Löhden et al. 1990).

In order to isolate the corresponding gene, we follow different strategies. First, we are trying to purify the enzyme. After successfull purification the gene for the LPA-AT can be identified by producing antibodies against the enzyme and screening of an expression cDNA gene bank of ripening seeds. As this approach is hindered by the fact that the enzyme is membrane bound and extremely labile after solubilization we additionally follow two other approaches. Both ways are termed unconventional in the sense that they do not require the purification of the enzyme but are based on the functional expression of the corresponding cDNA in microorganisms. To achieve this, mRNA is isolated from developing seeds of Limnanthes. From this mRNA pool full length cDNA is

synthesized, ligated into an expression vector and transferred into suitable host cells for expression. The two methods differ in the way to identify colonies containing cDNA for the LPA-AT.

On one hand, we are using a mutant strain of E.coli with a defective LPA-AT as a host (Coleman 1990). This block inhibits growth of the mutants under appropriate conditions. In some rare cases the expression vector might contain a functional copy of the plant LPA-AT cDNA leading to the production a functional enzyme. If this newly synthesized enzyme of plant origin encoded by the cDNA complements the missing enzyme activity of the mutant host strain, these particular cells will aquire the ability to grow even under restrictive conditions. Such complementation experiments have been successfully used to isolate a variety of genes (e.g. Lee and Nurse 1987, Koslow and Hill 1990; Delauny and Verma 1990).

The other method is based on a membrane assay. Here again a library made by shotgun cloning of cDNA from ripening seeds into an expression vector is used to transform microorganisms as host cells. After plating these cells onto agar plates replica filters are produced. The membrane bound permeabilized colonies are subsequently used to perform in situ enzyme assays. At the position of those colonies containing the desired enzyme activity a radioactively labelled soluble substrate is converted to an unsoluble precipitating product. After autoradiography the corresponding colonies on the original plate are identified. This type of approach has also been successfully carried out several times (Raetz 1975; Tillman and Bell 1986).

CONCLUSIONS

The block inhibiting a uniform esterification of all three positions of triacylglycerol from rapeseed oil with erucic acid has been correlated to the properties of one enzyme, namely LPA-AT. A plant containing an enzyme with the ability to introduce erucic acid into position 2 of the glycerol backbone has been identified. The isolation of a cDNA coding for this LPA-AT is currently in progress. This gene will be introduced into rapeseed plants and these transgenic varieties should lead to a higher proportion of erucic acid in rapeseed oil.

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