TRIERUCIN BIOSYNTHESIS IN TRANSGENIC RAPESEED: CLONING AND EXPRESSION OF cDNAs ENCODING AN ERUCOYL-CoA SPECIFIC ACYLTRANSFERASE

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ABSTRACT

From a seed-specific cDNA library of *Limnanthes douglasii* nine independent clones were selected which were able to complement an *E. coli* mutant defective in 1-acylglycerol-3-phosphate acyltransferase (1AGPAT). Six of those clones contained similar inserts. One of the clones (pCH21) was sequenced completely. It has a length of 1020 base pairs (bp) plus a polyA stretch and exhibits one long open reading frame encoding a polypeptide of 281 amino acids. The derived amino acid sequence shows substantial similarity to published 1AGPAT sequences. Expression studies in *E. coli* revealed 1AGPAT activity with a specificity for erucoyl-CoA.

INTRODUCTION

The industrial applicability of high erucic acid rapeseed oil could be improved appreciably by increasing the proportion of erucic acid to about 95% (Sonntag 1991). The main hindrance in this respect is the inability of rapeseed to accumulate trierucin in its oil. This is caused by the characteristics of the seed-specific 1AGPAT. Due to the pronounced specificity and selectivity of 1AGPAT for unsaturated C_{18} acyl groups, erucic acid is excluded from the *sn*-2 position of the glycerol backbone and, thus, the formation of trierucin is prevented (Bernerth and Frentzen 1990). In contrast, the respective seed-specific enzyme from *Limnanthes douglasii* effectively utilizes erucoyl-CoA as substrate (Löhden and Frentzen 1992). As a result of our strategy presented earlier to circumvent protein isolation by employing complementation (Wolter *et al.* 1991), here we report the isolation of a cDNA from *Limnanthes douglasii* encoding an erucoyl-CoA-specific 1AGPAT.

EXPERIMENTAL

Complementation with a seed-specific cDNA library

Ten days old embryos from *Limnanthes douglasii* were used to prepare cDNA and to construct an expression cDNA library in the vector lambda ZAP (Stratagene). The library, which contained 3.5×10^8 clones with an estimated average size for inserts of 1000 bp, was converted into the plasmid form and employed to complement the thermosensitive *E. coli* mutant JC201 with defective 1AGPAT activity (Coleman 1990). Nine clones could be selected which reproducibly allowed growth of the mutant under nonpermissive conditions. Six of them covered very similar inserts differing in their 5' ends only. One of the clones (pCH21) was further characterized.

Characteristics of pCH21

The cDNA insert of pCH21 has a length of 1020 bp plus additional 19 adenosine residues. It exhibits one long open reading frame of 852 bp in frame with the *lacZ* gene of the pBluescript vector (Stratagene). Thus, a fusion protein can be synthesized in *E. coli* being able to complement the enzymatic defect of the mutant. The first start codon is located at position 10 of the insert. As some other clones from the group of six exhibit a stop codon upstream to this start codon, it is assumed that this coding region represents the complete amino acid sequence of the *Limnanthes* gene with a length of 281 codons.

The amino acid sequence encoded by pCH21 showes substantial similarity to other sequences known or postulated to represent 1AGPAT (Table 1). Interestingly, the similarity between the two plant sequences from *Limnanthes* and maize is not higher than that between the ones from *Limnanthes* and yeast.

TABLE 1: Similarities and identities of amino acid sequences of known or postulated 1AGPATs of *E. coli* (Coleman 1992), yeast (Marek *et al.* 1993), maize (Brown *et al.* 1994), and *Limnanthes douglasii* (*Lim*). The sequence of *Salmonella typhimurium* (Luttinger *et al.* 1991) was omitted because it is more than 90% identical to the one of *E. coli*. Percentages of identities (upper right part) and similarities (lower left part) were calculated using the "bestfit" algorithm (Smith and Waterman 1981).

	E. coli	yeast	maize	*Lim*	
E. coli		31.4	24.8	30.0	
yeast	55.2		26.3	28.9	% identity
maize	50.0	53.9		27.7	
Lim	51.2	53.1	51.4		

% simlaity

Expression

The coding region of pCH21 was fused into the expression vector pQE60 (QIAGEN) leading to a construct termed pQEL21. Membrane fractions were isolated from *E. coli* JC201 cells harbouring either pQEL21 or pQE60, and assayed for 1AGPAT activity using oleoyl-CoA or erucoyl-CoA as acyl donor. Enzymatic activity was measured in membrane fractions derived from cells containing pQEL21 only. 1AGPAT activity determined with erucoyl-CoA was about three times higher than the one measured with oleyl-CoA. Hence, pCH21 encodes an erucoyl-specific 1AGAPT. Furthermore, Northern blot analyses showed that the gene corresponding to pCH21 is expressed in seeds but not in leaves. These data correlate well with the results of enzymatic studies, in which an erucoyl-CoA dependent 1AGPAT was detectable in developing seeds but not in leaves of *Limnanthes douglasii* (Löhden and Frentzen 1992).

Transformation

A chimeric gene was constructed by fusing the coding region of pCH21 to the promoter and polyadenylation signal of a rapeseed napin gene (Scofield and Crouch 1987).

The resulting construct was ligated into a binary *Agrobacterium* vector and used to transform spring rapeseed varieties with high erucic acid content. Probably we will be able to report on the establishing of trierucin biosynthesis and a concomitant increase in erucic acid content in the oil of such transgenic plants in near future.

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