CHARACTERIZATION OF THE PURIFIED $C_{18:1}$-COA ELONGASE FROM DEVELOPING RAPESEEDS (BRASSICA NAPUS L.).

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ABSTRACT :

The $C_{18:1}$-CoA elongase, the enzyme responsible for erucic acid synthesis, has been partially purified from developing rapeseeds using affinity, filtration and anion chromatographies. The elongase, presenting a molecular mass of 270 KDa, is composed at least of 3 different proteins of 61, 63 and 67 kDa. These results strongly suggest that the acyl-CoA elongase is a multi-enzyme complex.

INTRODUCTION :

Erucic acid is an important renewable material in oleochemical industries. In rapeseeds, erucic acid results from successive additions of malonyl-CoA to $C_{18:1}$-CoA carried out by a membrane-bound acyl-CoA elongase (Stumpf and Pollard, 1983, Créach et al. 1993). It has been proposed that the elongation process involves the following reactions : 1) condensation of acyl-CoA and malonyl-CoA leading to a 3-keto acyl-CoA, 2) reduction of the 3-keto acyl-CoA, 3) dehydratation of the 3-OH acyl-CoA and 4) reduction the of the trans 2-3 enoyl-CoA (Fehling and Mukherjee, 1991). In leek leaves, the acyl-CoA elongase has been solubilized and partially purified, showing the multi-enzymic nature of this enzyme (Bessoule et al. 1989). In oilseeds, the situation is not so advanced ; elongase activities have been solubilized from Sinapsis alba, Lunaria annua and Limnanthes alba (Murphy and Mukherjee 1989, Lardans and Tremolières 1992), and has been partially purified from L. annua (Fehling et al. 1992). The engineering research aiming to increase the erucic acid content of rapeseeds will require a better understanding of the acyl-CoA elongase from oilseeds. Using developing rapeseeds, a purification protocol has been elaborated and the acyl-CoA elongase has been partially purified.

EXPERIMENTAL

Purification of the acyl-CoA elongase

The acyl-CoA elongase activity, mainly associated with a 15 000 g membrane fraction, has been solubilized using a triton X-100 to protein ratio of 2.5. Under these conditions, 42 % of the proteins were recovered in the soluble fraction and the specific activity was 7.5-fold higher than that measured using the intact membrane enzyme. These proteins were subjected to different chromatographies that are summarized in Table 1. During the affinity chromatography on a C16-CoA agarose column, no activity was associated with the proteins which do not bind to the column. The acyl-CoA elongase was eluted using a 0.5 M NaCl step and presented an activity of 0.64 $nmol.mg^{-1}.h^{-1}$. During this step the phospholipid/protein ratio descreased from 0.63 to 0.37. Most of the proteins were retained on the filtration column, while the $C_{18:1}$-CoA elongase was recovered close to the void volume, suggesting an apparent molecular mass of 270 KDa for the acyl-CoA elongase embedded in a triton micelle. The fractions presenting the highest activities were pooled and subjected to DEAE-cellulose chromatography. The acyl-CoA elongase was recovered using a NaCl gradient in fractions eluting at 0.35 M NaCl. These fraction

contained 60 µg of proteins with a specific activity 5.3-fold higher than that of the 15 000 g pellet and were considerered as the partially purified enzyme.

Characterization of the purified acyl-CoA elongase :

- The oleoyl-CoA elongase activity was increased 5.3-fold during the purification process, compared to the activity measured in the intact membranes. Furthemore, in the presence of a mixture of PC (20 µg) and PE (5 µg), the C18:1 - CoA elongation was 2-fold higher than in the absence of lipids. These results suggest that the apparent purification factor is higher than that indicated in Table 1.
- The DEAE fraction containing the acyl-CoA elongase was analyzed by SDS-PAGE, which showed the presence of 3 major polypeptides of 61, 62 and 67 kDa, respectively.
- Immunoblotting experiments, using an anti-leek elongase antibody, showed that the antibodies raised against the leek elongase specifically recognize the proteins bands constituting the purified acyl-CoA elongase from rapeseeds.

In conclusion, these results show that the C18:1-CoA elongase, which has been partially purified from rapeseeds, was strongly delipidated and is composed of at least 3 different proteins, assuming that the acyl-CoA elongase is a multienzyme complex.

TABLE 1 : Purification of the C18 : 1 - CoA elongase from developing rapeseeds

Purification step	Specific Activity (nmol. mg $^{-1}$.h^{-1})	Phospholipids/ Proteins mass ratio	Apparent Purification factor
15 000 g pellet	0.19	1	1
Solubilized fraction	1.42	0.63	7.5
C_{16}-CoA agarose	0.64	0.37	3.4
Ultrogel AcA34	11.2	0.13	59
DEAE 52	1.0	<0.007	5.3
DEAE 52 + Lipids	2.13	-	11.3

The elongation activity was measured for each purified fraction (54 µl), using [2-^{14}C] malonyl-CoA (17 µM), oleoyl-CoA (9 µM) $MgCl_2$ (1mM), DTT (2 mM), NADPH (0.5 mM) and NADH (0.5 mM), in a 0.08 M Hepes buffer (pH 7.2), containing 10 mM ß-mercaptoethanol, 0.02 % Triton X - 100, 10 % ethylène glycol. The final volume was 0.1 ml and the incubation time was 1 h. The reaction was stopped by the addition of 100 µl of 20 % (w/v) KOH in H_2O : CH_3OH (9:1, v/v) and the fatty acids were extracted as described previously (Créach and Lessire, 1993).

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