The regulation of the fatty-acid composition of the triacylglycerols in microsomal preparations from avocado mesocarp and the developing cotyledons of safflower

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Abstract. The utilisation of [14C]glycerol 3-phosphate and [14C]linoleoyl-CoA in the synthesis of triacylglycerol has been studied in the microsomal preparations of developing cotyledons of safflower seed. The results confirm that the glycerol backbone, which flows towards triacylglycerol from phosphatidic acid through the Kennedy pathway, can enter phosphatidylcholine from diacylglycerol. The equilibration between diacylglycerol and phosphatidylcholine offers a mechanism for the return of oleate to phosphatidylcholine for desaturation to linoleate. We have established that the oleate entering position 1 of sn-phosphatidylcholine from diacylglycerol is desaturated in situ to linoleate. The results indicate that the diacylglycerol phosphatidylcholine interconversion coupled to the acyl exchange between acyl-CoA and position 2 of sn-phosphatidylcholine brings about the continuous enrichment of the glycerol backbone with C18-polyunsaturated fatty acids and hence these enzymes are of major importance in regulating the acyl quality of the accumulating triacylglycerols. Microsomal preparations from avocado mesocarp, however, did not have detectable acyl exchange between acyl-CoA and phosphatidylcholine or diacylglycerol phosphatidylcholine interconversion despite the high activity of the enzymes of the Kennedy pathway. A scheme is presented which incorporates many of the observations on triacylglycerol synthesis and provides a working model for the regulation of acyl quality in linoleate-rich vegetable oils.

Key words: Carthamus – Fatty acid (microsomes) – Microsome (triacylglycerol synthesis) – Persea –

Phosphatidylcholine – Seed (triacylglycerols) – Triacylglycerol.

Introduction
The control of the acyl composition of the triacylglycerols that accumulate in developing cotyledons of oil-rich seeds is little understood. Of particular importance are plant species and varieties that produce seed oils rich in C18-polyunsaturated fatty acids. It is now generally accepted that the synthesis of linoleic acid (C18:2) and, perhaps linolenic acid (C18:3), is by the sequential desaturation of the oleic acid (C18:1) that is associated with microsomal phosphatidylcholine (Stymne and Appelqvist 1978, 1980; Slack et al. 1979; Browse and Slack 1981).

We have previously shown that microsomal membranes of developing oil seeds (soy, safflower, sunflower) catalyze an acyl exchange between acyl-CoA and phosphatidylcholine (Stymne and Glad 1981; Stobart et al. 1982; Stymne and Stobart 1984a). We consider that the acyl exchange is of prime importance in the transfer of substrate to phosphatidylcholine for subsequent desaturation (Stymne et al. 1983). The products of desaturation are returned by further exchange to the acyl-CoA where they are utilised in the acylation of sn-glycerol 3-phosphate to yield phosphatidic acid and finally triacylglycerol (Stobart et al. 1983). Recently, we demonstrated that the acyl exchange is catalyzed by an acyl-CoA: lysophosphatidylcholine acyltransferase that can operate, in concert, in both a forward and back direction (Stymne and Stobart 1984b). Previous work with microsomal preparations from developing cotyledons of sunflower indicated that the glycerol backbone that flows
through the Kennedy pathway from phosphatidylcholine via diacylglycerol. No increase in mass of phosphatidylcholine occurred during the incorporation of glycerol and we proposed that an equilibration reaction catalysed by a cytidine 5'-diphosphate:1,2-diacylglycerol cholinephosphotransferase was probably involved (Stymne and Stobart 1984a; see also Slack 1983; Slack et al. 1983). The equilibration that develops between diacylglycerol and phosphatidylcholine when glycerol is moving to triacylglycerols may offer further opportunity to desaturate any oleate present in the diacylglycerol. The acyl exchange coupled to diacylglycerol phosphatidylcholine interconversion would, therefore, bring about the continuous enrichment of the glycerol backbone with polyunsaturated fatty acids.

The following report investigates the incorporation of glycerol and acyl-CoA in lipids in microsomal preparations of developing safflower cotyledons and determines whether the oleate entering the microsomal preparation to the reaction mixture. Bovine serum albumin was omitted from incubation mixtures and the fatty acids and lysophosphatidylcholine were also separated by TLC plates treated with silver nitrate as a fingerprint method that developes between the phosphatidylcholine, in the microsomal lipid extract, was purified by liquid chromatography using heptadecanoic acid as an internal standard. Monoenoic and dioenoic C18 fatty-acid methyl esters were also separated by TLC plates treated with silver nitrate as previously described (Stymne and Appelqvist 1980).

Positional analysis of fatty acids in phosphatidylcholine. Phosphatidylcholine, in the microsomal lipid extract, was purified by TLC and eluted from the gel in methanol/chloroform (4:1, v/v). The eluate was evaporated to dryness under N2 and the residue redissolved in 1 ml diethyl ether and 1 ml 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HC1 buffer, pH 8.9, containing 5 mM CaCl2 and phospholipase A2 (30 units). The mixture was incubated at 25°C for 30 min with constant shaking and the fatty acids and lysophosphatidylcholine extracted in water-saturated butanol-1-ol (Bjerve et al. 1974). After evaporation the residue was dissolved in chloroform and separated by TLC as for polar lipids.

Determination of radioactivity. Lipid samples were assayed for radioactivity in PCS® (Amersham/Seearle, Arlington Heights, USA), xylene (2:1) in a Beckman LS-230 liquid scintillation counter with an efficiency of 94% for 14C. Radioactivity in samples of silver-nitrate gel containing the methyl esters of oleic and linoleic acid were assayed in 2,5-diphenyloxazole-phenyloxazolylphenyloxazolylphenyl (PPO-POPOP) in toluene (4 g PPO + 0.3 g POPOP 1-1 (toluene). All counts were corrected for background and quenching.

Results

The utilisation of [14C]sn-glycerol 3-phosphate in the presence of linoleoyl-CoA in safflower microsomal preparations. Microsomal preparations (equivalent to 150 nmol phosphatidylcholine) were incubated with [14C]sn-glycerol 3-phosphate (200 nmol) in the presence of linoleoyl-CoA (250 nmol). The radioactivity incorporated into various lipids was determined at regular intervals. The results (Fig. 1) show that [14C] glycerol was rapidly incorporated into phosphatidic acid and reached a...