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The utilisation of fatty-acid substrates in triacylglycerol biosynthesis by tissue-slices of developing safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) cotyledons

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Abstract. Developing cotyledons of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) readily utilised exogenously supplied ^{14}C-labelled fatty-acid substrates for the synthesis of triacylglycerols. The other major radioactive lipids were phosphatidylcholine and diacylglycerol. In safflower cotyledons, [^{14}C]oleate was rapidly transferred to position 2 of *sn*-phosphatidylcholine and concomitant with this was the appearance of radioactive linoleate. The linoleate was further utilised in the synthesis of diacyl- and triacyl-glycerol via the reactions of the so-called Kennedy pathway. Supplying [^{14}C]linoleate, however, resulted in a more rapid labelling of the diacylglycerols than from [^{14}C]oleate. In contrast, sunflower cotyledons readily utilised both labelled acyl substrates for rapid diacylglycerol formation as well as incorporation into position 2 of *sn*-phosphatidylcholine. In both species, however, [^{14}C]palmitate largely entered *sn*-phosphatidylcholine at position 1 during triacylglycerol synthesis. The results support our previous in-vitro observations with isolated microsomal membrane preparations that (i) the entry of oleate into position 2 of *sn*-phosphatidylcholine, via acyl exchange, for desaturation to linoleate is of major importance in regulating the level of polyunsaturated fatty acids available for triacylglycerol formation and (ii) Palmitate is largely excluded from position 2 of *sn*-phosphatidylcholine and enters this phospholipid at position 1 probably via the equilibration with diacylglycerol. Specie differences appear to exist between safflower and sunflower in relation to the relative importance of acyl exchange and the interconversion of diacylglycerol with phosphatidylcholine as mechanisms for the entry of oleate into the phospholipid for desaturation.

Key words: *Carthamus* – Fatty acids – *Helianthus* – Lipid biosynthesis – Triacylglycerol.

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Abbreviations: FW = fresh weight; TLC = thin-layer chromatography

Introduction

Recently we have proposed models (Stobart and Stymne 1985a; Stymne and Stobart 1987) to explain the acyl quality of linoleate (C-18:2)-rich seed oils (Stymne and Stobart 1984a; Stymne et al. 1983). Our present understanding is briefly illustrated in Fig. 1. The desaturation of oleate (C-18:1) to linoleate (C-18:2) occurs at position 2 of microsomal *sn*-phosphatidylcholine (Stymne and Appelqvist 1978; Slack et al. 1979). A major problem is to account for the entry of oleate into phosphatidylcholine and to establish how the desaturated products are made available for the assembly of the triacylglycerols. It has been suggested (Slack et al. 1978, 1983) that phosphatidylcholine may give rise to polyunsaturated species of diacylglycerol which are further acylated to yield triacylglycerols. We have shown, however, in microsomal preparations, that the oleate in oleoyl-CoA equilibrates with linoleate in position 2 of *sn*-phosphatidylcholine (Stymne et al. 1983; Stobart et al. 1983; Stymne and Stobart 1984b). This equilibration returns linoleate to the acyl-CoA pool where it is selectively utilized in the acylation of *sn*-glycerol 3-phosphate to yield phosphatidate (Stymne et al. 1983; Stobart et al. 1983) which is then converted to diacyl- and triacyl-glycerol by the reactions of

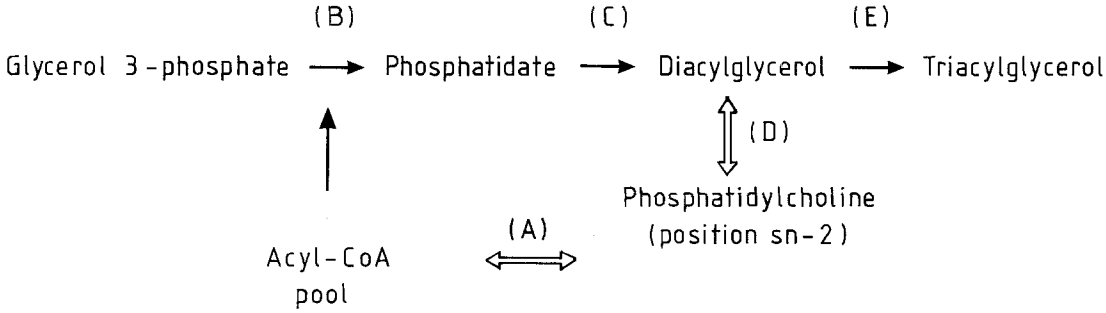

Fig. 1. Scheme showing the pathway of C_{18}-polyunsaturated fatty-acid-rich triacylglycerol formation in developing oilseeds. Acyl exchange (*A*) controls, principally, the movement of oleate to position 2 of *sn*-phosphatidylcholine for desaturation. The linoleate so formed can be returned to the acyl-CoA pool by way of the back-reaction of an acyl-CoA:lysophosphatidylcholine acyltransferase, and from there, is utilized together with oleate, (and also saturated acyl species) in the two-step acylation of *sn*-glycerol 3-phosphate to form phosphatidic acid (*B*). The phosphatidic acid is then converted to diacylglycerol (*C*) which can be re-cycled through the phosphatidylcholine pool (*D*) for further opportunities at the desaturation of its constituent oleate groups. Ultimately, the diacylglycerols are acylated to form triacylglycerol (*E*)

the so-called Kennedy pathway. An interconversion of diacylglycerol with phosphatidylcholine also occurs when glycerol backbone flows from phosphatidate to triacylglycerol (Stobart and Stymne 1985b) and this may be catalysed by a cholinephosphotransferase (Slack et al. 1978, 1983). The interconversion of phosphatidylcholine and diacylglycerol may give further opportunities to desaturate oleate and so bring about some enrichment of the glycerol backbone with C-18-polyunsaturated fatty acids (Stobart and Stymne 1985a). In studies on the acylation of *sn*-glycerol 3-phosphate in safflower microsomes we have shown (Griffiths et al. 1985) that the saturated fatty acids, stearate (C-18:0) and palmitate (C-16:0), were almost exclusively utilised in the esterification of position 1 whereas linoleate was largely used at the *sn*-2 position. Oleate on the other hand, showed a preference for position *sn*-1. The acyl specificity and selectivity properties of the enzymes involved in the acylation of *sn*-glycerol 3-phosphate, therefore, result in the stereospecific distribution of acyl components at positions 1 and 2 of *sn*-phosphatidate and subsequently governs the non-random distribution of the fatty-acid constituents observed in the seed triacylglycerols (Hilditch and Williams 1964; Ichihara and Noda 1980). The present report is an attempt to reconcile our in-vitro observations with in-vivo studies on fatty-acid utilisation and lipid assembly, and deals with the metabolism of acyl species in the developing cotyledon tissue from safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.).

Material and methods

Materials. Safflower (*Carthamus tinctorius*, cv. Very High-Linoleate, (VHL)) and sunflower (*Helianthus annuus* L., cv. Giant Yellow) were grown from seed in a 16-h photoperiod at a temperature of 28–32° C and an 8-h night with a minimum temperature of 20° C. 1-^{14}C-Labelled fatty-acids were obtained from Amersham International (Amersham, Bucks., UK). Phospholipase A_2 (Indian cobra venom) was obtained from the Sigma Chemical Co. (St. Louis, Mo., USA). 1-^{14}C oleate (Δ^9-18:1; specific activity 2.15 GBq·mmol^{-1}), [1-^{14}C]linoleate ($\Delta^{9,12}$-18:2; specific activity 2.15 GBq·mmol^{-1}) and [1-^{14}C]palmitate (16:0; specific activity 2.07 GBq·mmol^{-1}) were used as their ammonium salts.

Tissue preparation. Developing cotyledons were harvested from the oilseeds 14–18 d after flowering at a time corresponding to maximal rates of triacylglycerol deposition (Slack et al. 1985), and used within 0.5–1 h following excision. The experiments reported were performed on at least two occasions with different batches of seed material.

Incubation reaction mixtures. Incubations were carried out in testtubes at 30° C with constant shaking. Each incubation contained approx. 0.3 g fresh weight (FW) of thinly sliced cotyledonary tissue and radioactive fatty-acid substrate (equilivant to 74 kBq) in a total volume of 1 ml of potassium-phosphate buffer, pH 7.2. All of the ammonium salts of the ^{14}C-fatty acids were rapidly taken up by the tissues over the first 80 min of incubation, after which the level of radioactivity in the lipid fraction remained almost constant. The uptake of [^{14}C]acyl substrates (as ammonium salts) was comparable for both species.

Analytical procedures. Radiolabelled cotyledons were rinsed with distilled water (3 × 3-ml portions) prior to lipid extraction to remove exogenous isotope. Lipids were extracted in chloroform/methanol/0.15 M acetic acid (10:20:7.5, by vol.) followed by the further addition of chloroform (10 ml) and water (10 ml; modified from Bligh and Dyer 1959). The chloroform phase was reduced to dryness under N_2 and the residue dissolved in a small volume of chloroform. The polar and neutral lipids were purified by thin-layer chromatography (TLC) on precoated silica-gel plates (Silica gel 60; Merck, Darmstadt, FRG) with chloroform/methanol/acetic acid/water (170:30:20:7, by vol.) and hexane/diethylether/acetic acid (70:30:1, by vol.), respectively. Lipids were located on the TLC plates by lightly staining with I_2 vapour and then removed from the plates for radioassay or transmethylation for the analysis of fatty-acids. The fatty-acid methyl esters were prepared in methanol-HCl and quantified by gas-liquid chromatography using heptadec-

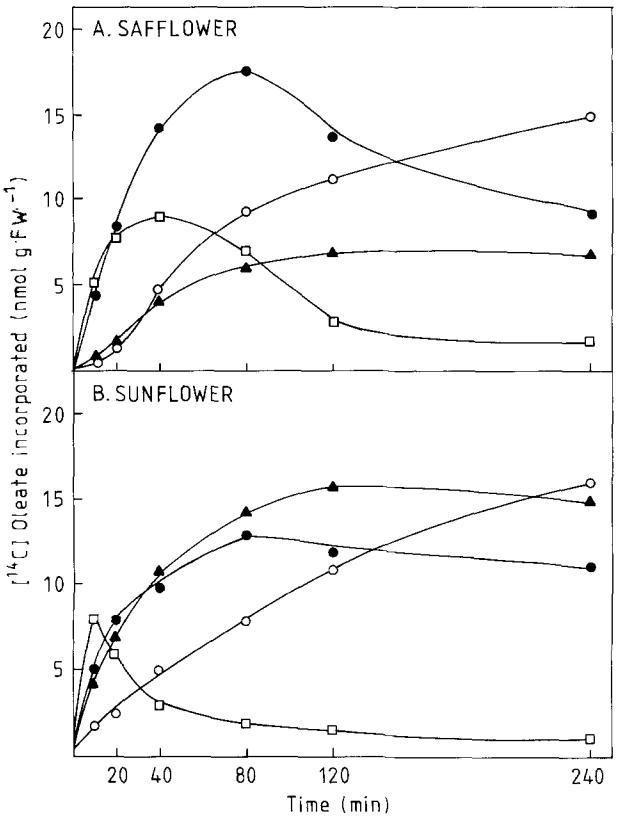

Fig. 2A, B. The utilisation of [^{14}C]oleate by tissue slices from developing cotyledons of (**A**) safflower and (**B**) sunflower. The tissues, (equivalent to 0.3 g FW portions) were incubated in phosphate buffer (1 ml) containing 74 kBq [^{14}C]oleate. Lipids were extracted and purified from the tissues at regular intervals and radioassayed. ●—●, phosphatidylcholine; □—□, unesterified fatty acid; ▲—▲, diacylglycerol; ○—○, triacylglycerol

anoic acid as the internal standard (Stymne and Stobart 1985). Monoenoic and dienoic C18 fatty-acid methyl esters were separated by argentation-TLC using a double development in petroleum ether (boiling range 40–60° C)/diethylether (95:5, v/v). Methyl esters were detected under ultraviolet light after spraying with 2,7-dichlorofluorescein (0.2% w/v in ethanol).

Positional analysis of fatty-acids in phosphatidylcholine. Phosphatidylcholine was purified by TLC and eluted from the gel in methanol/chloroform (2:1, v/v). The eluate was evaporated to dryness under N_2 and the residue resuspended in 1 ml diethylether and 1 ml 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl, pH 8.9, containing 5 mM $CaCl_2$ and phospholipase A_2 (30 units). After incubation at 25° C for 30 min the fatty-acids and lysophosphatidylcholine were extracted in water-saturated butan-1-ol (Bjerve et al. 1974) and separated by TLC as for polar lipids.

Determination of radioactivity. Lipid samples were assayed for radioactivity in PCS (Amersham/Searle, Arlington Heights, Ill., USA), xylene (2:1 by vol.) in an Isocap 300 Nuclear Chicago liquid scintillation counter with an efficiency of 80% for ^{14}C. 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 (4g PPO + 0.3 g POPOP/L-toluene). All counts were corrected for quenching.

Table 1. Positional distribution of radioactivity in *sn*-phosphatidylcholine from developing oilseed cotyledons incubated with [^{14}C]oleate. The phosphatidylcholine from the time-course studies with safflower (**Fig. 2a**) and sunflower (**Fig. 2b**) was purified and treated with phospholipase A_2. Figures in ( ) are the relative distributions of radioactivity in *sn*-phosphatidylcholine

Incubation time (min)	*sn*-1 (nmol·g FW^{-1})	*sn*-2 (nmol·g FW^{-1})
Safflower		
10	0.3 (7)	4.3 (93)
20	0.5 (6)	7.5 (94)
80	1.8 (10)	15.7 (90)
240	1.6 (17)	7.6 (83)
Sunflower		
10	1.1 (21)	4.0 (79)
40	2.5 (25)	7.3 (75)
120	2.4 (21)	9.1 (79)
240	2.0 (19)	8.6 (81)

Results

Utilisation of [^{14}C]oleate in safflower and sunflower. Safflower cotyledon slices were incubated with [^{14}C]oleate and at regular intervals the radioactivity taken up and incorporated into the lipids was determined. The ^{14}C in the total lipid fraction increased at an almost linear rate for the first 80 min incubation and then remained at an essentially similar level throughout the remainder of the experiment. The distribution of radioactivity in the individual lipids (Fig. 2a) showed that the [^{14}C]oleate readily accumulated in the complex lipids. Phosphatidylcholine was the first lipid in which the oleate became associated and its radioactivity increased up to 80 min followed by a rapid decline for the remainder of the incubation period. Diacylglycerols were labelled less extensively than phosphatidylcholine and at a somewhat slower rate. Radioactivity in triacylglycerol accumulated throughout the experiment until it represented some 40% of the total activity incorporated. Transmethylation of the total lipid fraction indicated that over 95% of the radioactivity resided in the acyl moieties (data not shown). The intramolecular distribution of the radioactivity in *sn*-phosphatidylcholine shows that initially some 94% of the label was located at position 2 and that this steadily declined to about 80% at the end of the incubation (Table 1). To assess the efficiency of the desaturation of oleate in the tissues an analysis of the radioactivity present in the oleoyl and linoleoyl moieties in the lipids was carried out (Table 2). After only 10 min incubation some 25% of the total radioactivity was now attributable to lino-

Table 2. Relative distribution of radioactivity in the oleoyl-, and linoleoyl-moieties of the major lipids in oilseed cotyledons incubated with [^{14}C]oleate. The major lipids, phosphatidylcholine, diacylglycerol and triacylglycerol were purified and transmethylated using methanolic-HCl. The fatty-acid methyl esters were purified using silver-nitrate TLC and assayed for radioactivity

Incubation time (min)	Phosphatidyl choline		Diacyl-glycerol		Triacyl-glycerol	
	18:1	18:2	18:1	18:2	18:1	18:2
Safflower						
10	74	26	74	26	66	34
20	64	36	72	28	60	40
80	45	55	44	56	39	61
240	32	68	30	70	29	71
Sunflower						
10	94	6	91	9	88	12
240	72	28	82	18	77	23

leate and after 4 h this amounted to over 70%. The proportions of radioactivity in the oleate and linoleate of phosphatidylcholine, diacylglycerol and triacylglycerol followed a similar trend, although slightly higher levels of [^{14}C]linoleate were present initially in the latter lipid. The extent of [^{14}C]oleate desaturation was also determined at both positions 1 and 2 of *sn*-phosphatidylcholine. The results (Table 3) indicate that initially oleate was the major labelled acyl component at both positions 1 and 2 of *sn*-phosphatidylcholine. In view of the larger mass of label at position 2 (see Table 1), 93% of the total [^{14}C]oleate in phosphatidylcholine was present at this position. A similar value was found for [^{14}C]linoleate after 10 min incubation. On a mass basis these figures represent some 14 times more oleate and 17 times more linoleate at position 2 than at position 1. With time the relative proportion of [^{14}C]linoleate at both positions increased to a similar extent (Table 3) and after 4 h incubation amounted to nearly 70% of the [^{14}C]acyl residues at both positions 1 and 2 of the *sn*-phosphatidylcholine. Calculations based on the decrease in the absolute amounts of the [^{14}C]fatty-acids from phosphatidylcholine, which occurred between 80 and 240 min incubation (Table 1), show that over 40 times more radioactivity was lost from position 2 than from position 1.

Similar experiments were conducted with sunflower cotyledons (Fig. 2b and Tables 1 and 2). In contrast to safflower, the labelling kinetics of the complex-lipids (Fig. 2b) show that phosphatidylcholine and diacylglycerol were labelled at a similar rate for 80 min after which it declined

Table 3. Positional distribution of [^{14}C]oleate and [^{14}C]linoleate in safflower *sn*-phosphatidylcholine after incubation of the cotyledons in [^{14}C]oleate substrate. The phosphatidylcholine from the experiment described in **Fig. 2a** was purified and hydrolysed using phospholipase A_2. Radioactivity in the oleoyl- and linoleoyl-moieties was determined following silver-nitrate TLC of the fatty-acid methyl esters. nmol = nmol ^{14}C fatty-acid at each position · g FW^{-1}

Incubation time (min)	Incorporation into position:							
	sn-1				*sn*-2			
	18:1		18:2		18:1		18:2	
	nmol	%	nmol	%	nmol	%	nmol	%
10	0.24	80	0.06	20	3.26	76	1.04	24
20	0.34	68	0.16	32	4.78	64	2.72	36
80	0.90	50	0.90	50	7.16	46	8.54	54
240	0.49	31	1.11	69	2.49	33	5.11	67

Table 4. Fatty-acid composition of the major acyl lipids in the developing cotyledons of safflower and sunflower. The results are a mean ± SD for three separate analyses

	Fatty-acid (mol %)			
	16:0	18:0	18:1	18:2
Safflower				
Phosphatidylcholine	10±1	4±1	3±1	83±2
Diacylglycerol	8±1	5±1	4±1	83±2
Triacylglycerol	6±1	2±0	5±1	87±2
Sunflower				
Phosphatidylcholine	9±1	4±1	16±1	71±1
Diacylglycerol	7±1	1±0	22±2	70±3
Triacylglycerol	5±0	2±0	30±2	63±2

slightly in phosphatidylcholine and carried on accumulating in diacylglycerol. At the end of the incubation period the diacylglycerol and triacylglycerol were almost equally labelled. Positional analysis of the distribution of radioactivity in *sn*-phosphatidylcholine (Table 1) showed that some 80% of the label was associated with position 2 throughout the time-course. The rate of oleate desaturation in the sunflower cotyledons was relatively low (Table 2) compared to safflower and even after 4 h incubation only 28% of the radioactivity in phosphatidylcholine was present in linoleate.

The differences in [^{14}C]oleate utilisation observed in the two species was also reflected in the acyl composition of the major lipids (Table 4). The acyl compositions of the diacylglycerol and phosphatidylcholine from safflower were almost identical whereas, in sunflower, the diacylglycerol was relatively richer in oleate. Similarly, the sunflower

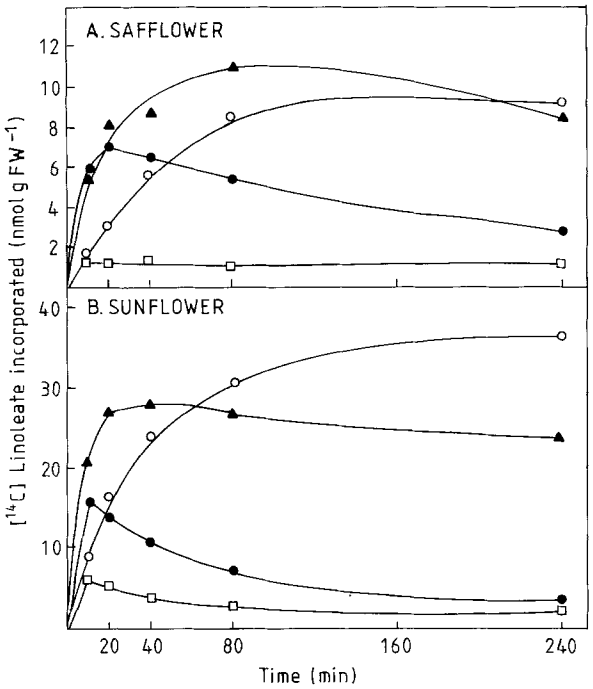

Fig. 3A, B. The utilisation of [^{14}C]linoleate by the developing cotyledons of (**A**) safflower and (**B**) sunflower. Tissue slices of the oilseed cotyledons were incubated in phosphate buffer (1 ml) containing 74 kBq [^{14}C]linoleate. At regular intervals the lipids were extracted from the tissues and after purification assayed for radioactivity. ●—●, phosphatidylcholine; □—□, unesterified fatty acid; ▲—▲, diacylglycerol; ○—○, triacylglycerol

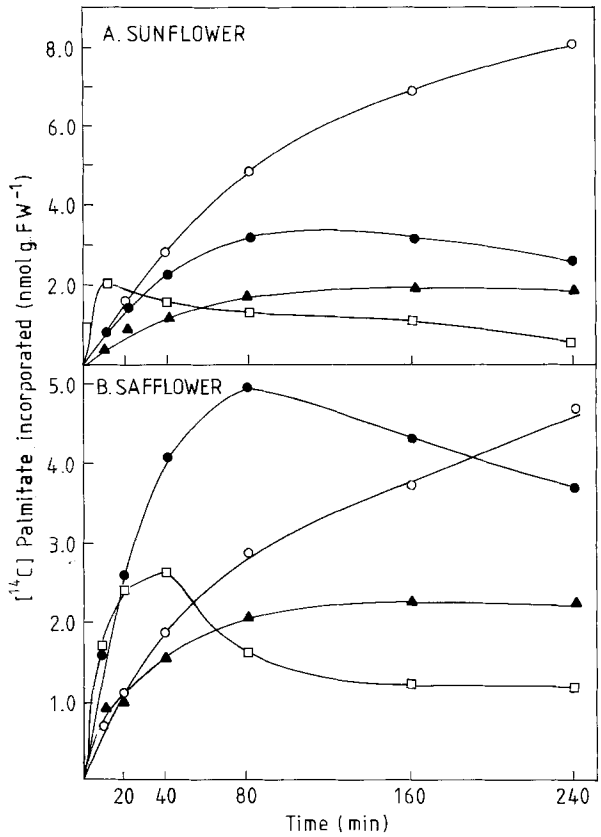

Fig. 4A, B. The utilisation of [^{14}C]palmitate by developing cotyledons of (**A**) sunflower and (**B**) safflower. Tissue slices of the oilseeds were incubated in phosphate buffer (1 ml) containing 74 kBq [^{14}C]palmitate. At regular intervals the tissues were extracted and the lipids assayed for radioactivity. ●—●, phosphatidylcholine; □—□, unesterified fatty acid; ▲—▲, diacylglycerol; ○—○, triacylglycerol

phosphatidylcholine contained relatively higher proportions of oleate than safflower.

Utilisation of [^{14}C]linoleate. Safflower microsomal preparations utilise linoleate preferentially for phosphatidate synthesis (Stobart et al. 1983; Griffiths et al. 1985). If a similar situation occurs in vivo then the diacylglycerols should become more extensively labelled with [^{14}C]linoleate (i.e. glycerol 3-phosphate + linoleate → phosphatidate → diacylglycerol) than in the previous experiments with [^{14}C]oleate. Slices of safflower cotyledons were, therefore, incubated with [^{14}C]linoleate and its distribution determined in the major lipids. The results (Fig. 3a) show that phosphatidylcholine and diacylglycerol were labelled to an almost identical extent over the first 10 min of incubation. However, by 20 min, diacylglycerol was the major radioactive lipid and after this time continued to accumulate label up to 80 min and then decreased. The [^{14}C]linoleate in phosphatidylcholine steadily declined after 20 min and by 4 h had lost some 60% of its label. Triacylglycerols, on the other hand, continued to increase in radioactivity throughout the experiment. Similar studies with sunflower again showed that over the early periods of incubation the diacylglycerols were the most radioactive of the major complex-lipids (Fig. 3b).

An analysis of the intramolecular distribution of [^{14}C]linoleate in the *sn*-phosphatidylcholine of safflower and sunflower showed that initially, some 90% of the [^{14}C]linoleate was esterified to position 2 and that this declined slightly with time (data not shown).

Utilisation of [^{14}C]palmitate. In-vitro studies (Ichihara 1984; Griffiths et al. 1985) indicate that palmitate will be utilised, largely, in the acylation of position 1 of *sn*-glycerol 3-phosphate. The formation of diacylglycerol from phosphatidate and the subsequent interconversion of diacylglycerol with phosphatidylcholine should result, therefore, in the entry of palmitate at position 1 of the latter lipid. A study of the utilisation of [^{14}C]palmitate in the developing cotyledons of sunflower (Fig. 4a) showed that over the first 20 min of incubation

Table 5. Positional distribution of radioactivity, in *sn*-phosphatidylcholine from developing oilseed cotyledons incubated with [^{14}C]palmitate

Incubation time (min)	Incorporation into position:			
	sn-1		*sn*-2	
	nmol·g FW^{-1}	%	nmol·g FW^{-1}	%
Sunflower				
10	0.6	75	0.2	25
80	2.6	81	0.6	19
240	2.2	85	0.4	15
Safflower				
10	1.0	63	0.6	37
80	3.8	76	1.2	24
240	3.0	81	0.7	19

the phosphatidylcholine and triacylglycerol were labelled at an essentially similar rate. After 20 min, however, the triacylglycerol became the most radioactive complex-lipid. Initially, 75% of the [^{14}C]palmitate which entered *sn*-phosphatidylcholine was present at position 1 (Table 5) and this increased to some 85% during the incubation period.

Experiments with safflower (Fig. 4b), however, showed a marked difference in the labelling pattern of the complex-lipids compared to sunflower (Fig. 4a). The incorporation of [^{14}C]palmitate into phosphatidylcholine was particularly rapid over the first 80 min and, clearly, was the most heavily labelled lipid over this time. After 80 min, however, there was a decrease of some 25% in the radioactivity in phosphatidylcholine. Triacylglycerol, however, continued to accumulate label throughout the experiment. It is evident (Table 5) that, initially a substantial proportion of [^{14}C]palmitate was present at position 2 of *sn*-phosphatidylcholine (some 37%). With time, however, the proportion of radioactivity at position *sn*-1 increased.

Discussion

Exogenously supplied oleate, linoleate and palmitate were efficiently utilised in lipid synthesis during triacylglycerol formation in the developing cotyledons of safflower and sunflower. The fatty-acid substrates were rapidly and principally incorporated into the acyl-groups of phosphatidylcholine, diacylglycerol and triacylglycerol. The substantial labelling of diacylglycerol in safflower from [^{14}C]linoleate (Fig. 3a) and sunflower from [^{14}C]oleate (Fig. 2b) and linoleate (Fig. 3b) indicate that the third acylation step in triacylglycerol biosynthesis (catalysed by a diacylglycerol acyltransferase) could be, to some extent, rate-limiting. Extensive labelling of diacylglycerol was also observed in soya and linseed (Dybing and Craig 1970; Slack et al. 1978) and this may reflect a flux of diacylglycerol through phosphatidylcholine before entering triacylglycerol (Slack et al. 1983, 1985; Stobart and Stymne 1985b). The utilisation of [^{14}C]oleate in safflower and sunflower (Fig. 2a, b) indicates that position 2 of *sn*-phosphatidylcholine was extensively labelled and in conjunction with this the desaturation of oleate to linoleate occurred. The substantial labelling of phosphatidylcholine with [^{14}C]oleate, in advance of the other lipids, together with the rapid appearance of [^{14}C]linoleate is to be expected since this lipid is considered the substrate for oleate desaturation (Gurr et al. 1969; Stymne and Appelqvist 1978; Slack et al. 1979; Murphy and Stumpf 1980; Wilson et al. 1980). The [^{14}C]linoleate, principally from position 2 of *sn*-phosphatidylcholine (Table 3), increased in diacylglycerol and triacylglycerol (Table 2) indicating the movement of the polyunsaturated component from the phospholipid to these lipids. The rapid turnover of acyl species at position 2 of *sn*-phosphatidylcholine would occur if acyl exchange was operating between the acyl-CoA pool and this position (Stymne and Glad 1981; Stymne et al. 1983). The appearance of [^{14}C]linoleate at position 1 of *sn*-phosphatidylcholine could have occurred, however, by (i) the in-situ desaturation of oleate at position 1 as observed in vitro (Slack et al. 1979; Stobart and Stymne 1985a) and/or (ii) its re-entry at position 1 through the Kennedy pathway. It is evident from the labelling kinetics of diacylglycerol (see Fig. 2a, b) and from the acyl composition of endogenous acyl lipids (Table 4) that oleate is utilised differently in safflower and sunflower. In the latter species it appears from the rapid initial labelling of the diacylglycerols that oleate and linoleate are utilised by the *sn*-glycerol 3-phosphate-acylating enzymes. In safflower, however, oleate is more rapidly transferred to position 2 of *sn*-phosphatidylcholine for desaturation leaving little oleate for the acylation of *sn*-glycerol 3-phosphate and hence the lag in the labelling of diacylglycerol. Supplying [^{14}C]linoleate to safflower resulted in the extensive labelling of the diacylglycerols as well as phosphatidylcholine over the initial time periods (Fig. 3a), in contrast with [^{14}C]oleate (Fig. 2a) where phosphatidylcholine was the major labelled lipid. Two explanations exist for the rapid rate of diacylglycerol labelling from [^{14}C]linoleate: (i) linoleate is used in preference to oleate in the acylation of *sn*-

glycerol 3-phosphate as reported in vitro (Stobart et al. 1983; Griffiths et al. 1985) and (ii) the incorporation of linoleoyl moieties into position 2 of *sn*-phosphatidylcholine results in a preferential utilisation of this molecular species in the back-reaction of a cholinephosphotransferase. Little, or no, selectivity towards molecular species of phosphatidylcholine or diacylglycerol has been reported, however, for the cholinephosphotransferase in oilseeds (Slack et al. 1983, 1985). The extensive labelling of position 2 of *sn*-phosphatidylcholine with [^{14}C]linoleate is consistent with the entry of this acyl group via acyl exchange and the diacylglycerol interconversion with phosphatidylcholine.

The results with [^{14}C]palmitate indicate that position 1 of *sn*-phosphatidylcholine is more extensively labelled with saturated acyl groups than in the previous experiments using unsaturated acyl substrates. In vitro, saturated acyl-CoAs (palmitate and stearate) have been shown exclusively to acylate position 1 of *sn*-glycerol 3-phosphate (Griffiths et al. 1985) and this coupled to the diacylglycerol-phosphatidylcholine interconversion (Slack et al. 1983; Stobart and Stymne 1985b) would ensure the entry of these acyl components at position 1 of *sn*-phosphatidylcholine. In safflower, however, palmitate entered position 2 of *sn*-phosphatidylcholine in the early periods of incubation (Table 5). Palmitate may, therefore, be utilised by the acyl-exchange, between the acyl-CoA pool and position 2 of *sn*-phosphatidylcholine, under conditions in which the concentration of competing C-18-unsaturated fatty-acids is relatively low (see Griffiths et al. 1985). The observations that palmitate is almost totally excluded from position 2 in safflower *sn*-triacylglycerol (Ichihara and Noda 1980) and constitutes only a negligible proportion of the acyl complement at this position in phosphatidylcholine (Griffiths et al. 1985) indicates that under in-vivo conditions, the proportion of unsaturated fatty-acids (particularly linoleate) in the acyl-CoA pool, is high. The differences observed between sunflower and safflower, with respect to palmitate, may reflect differences in the acyl-selectivity properties of the *sn*-glycerol 3-phosphate-acylating enzymes and the acyl-CoA:lysophosphatidylcholine acyltransferase in the two species. Such specie differences may be exaggerated by a differential rate of acyl exchange relative to the acylation of *sn*-glycerol 3-phosphate. In spite of this, however, it is evident that the palmitate enters phosphatidylcholine from diacylglycerol via the Kennedy pathway. The relatively rapid labelling of triacylglycerol from [^{14}C]palmitate in both species (Fig. 4a, b) may also reflect a rapid acylation to position 3 of endogenous *sn*-1,2-diacylglycerols and this could account for the relatively high levels of this acyl component at position 3 in the seed *sn*-triacylglycerols (Hilditch and Williams 1964; Ichihara and Noda 1980).

The results presented give further support to our previous proposals (Fig. 1) that were largely based on in-vitro systems (see Stobart and Stymne 1985a; Griffiths et al. 1985; Stymne and Stobart 1987), namely (i) saturated acyl groups are selectively utilised for acylating position 1 of *sn*-glycerol 3-phosphate and are selected against in the acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine (saturated components therefore, enter position 1 of *sn*-phosphatidylcholine by the interconversion of diacylglycerol and phosphatidylcholine during the operation of the Kennedy pathway) and (ii) oleate enters position 2 of *sn*-phosphatidylcholine by a mechanism which is not inconsistent with the operation of an acyl exchange between acyl-CoA and phosphatidylcholine. The high turnover of unsaturated fatty-acids at position 2 of *sn*-phosphatidylcholine, coupled to the desaturation of oleate to linoleate, indicates that the acyl exchange, which only operates at this position (Stymne et al. 1983), is relatively of major importance in regulating the acyl quality. The equilibration of diacylglycerol and phosphatidylcholine during triacylglycerol synthesis appears to be of lesser importance in the synthesis of linoleate and the control of the final level of unsaturation of the seed-oil. In sunflower, however, the extensive labelling of diacylglycerols with oleate would indicate that the diacylglycerol-phosphatidylcholine interconversion may be of greater importance at increasing the level of linoleate in the triacylglycerol. Mechanisms for the continuous re-cycling of oleate to phosphatidylcholine could, therefore, be of greater importance in species with lower rates of oleate desaturation.

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References

Bjerve, K.S., Daae, L.N.W., Bremer, J. (1974) The selective loss of lysophospholipids in some commonly used lipid-extraction procedures. Anal. Biochem. **58**, 238–245

Bligh, E.G., Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**, 911–917

Dybing, C.D., Craig, B.M. (1970) Fatty acid biosynthesis and incorporation into lipid classes in seeds and seed tissues of flax. Lipids **5**, 422–429

Griffiths, G., Stobart, A.K., Stymne, S. (1985) The acylation of *sn*-glycerol 3-phosphate and the metabolism of phosphatidate in microsomal preparations from the developing cotyledons of safflower (*Carthamus tinctorius* L.) seed. Biochem. J. **230**, 379–388

Gurr, M.I., Robinson, M.P., James, A.T. (1969) The mechanism of formation of polyunsaturated fatty acids by photosynthetic tissue. Eur. J. Biochem. **9**, 70–78

Hilditch, T.P., Williams, P.N. (1964) The chemical constitution of natural fats. Chapman and Hall, London

Ichihara, K. (1984) *sn*-Glycerol 3-phosphate acyltransferase in a particulate fraction from maturing safflower seeds. Arch. Biochem. Biophys. **232**, 685–698

Ichihara, K., Noda, M. (1980) Fatty acid composition and lipid synthesis in developing safflower seeds. Phytochemistry **19**, 49–54

Murphy, D.J., Stumpf, P.K. (1980) In vivo pathway of oleate and linoleate desaturation in developing cotyledons of *Cucumis sativus* L. seedlings. Plant Physiol. **66**, 666–671

Slack, C.R., Campbell, L.C., Browse, J.A., Roughan, P.G. (1983) Some evidence for the reversibility of the cholinephosphotransferase-catalyzed reaction in developing linseed cotyledons in-vivo. Biochim. Biophys. Acta **754**, 10–20

Slack, C.R., Roughan, P.G. Balasingham, N. (1978) Labelling of glycerolipids in the cotyledons of developing oilseeds by 1-^{14}C acetate and 2-^{3}H glycerol. Biochem. J. **170**, 421–433

Slack, C.R., Roughan, P.G., Browse, J. (1979) Evidence for an oleoylphosphatidylcholine desaturase in microsomal preparations from cotyledons of safflower seeds. Biochem. J. **179**, 649–656

Slack, C.R., Roughan, P.G., Browse, J.A., Gardiner, S.E. (1985) Some properties of cholinephosphotransferase from developing safflower cotyledons. Biochim. Biophys. Acta **833**, 438–448

Stobart, A.K., Stymne, S. (1985a) The regulation of the fatty-acid composition of the triacylglycerols in microsomal preparations from avocado mesocarp and the developing cotyledons of safflower. Planta **163**, 119–125

Stobart, A.K., Stymne, S. (1985b) The interconversion of diacylglycerol and phosphatidylcholine during triacylglycerol production in microsomal preparations of developing cotyledons of safflower (*Carthamus tinctorius* L.). Biochem. J. **232**, 217–221

Stobart, A.K., Stymne, S., Glad, G. (1983) The synthesis of linoleate and phosphatidic acid and its relationship to oil production in the microsomes of developing seeds of safflower (*Carthamus tinctorius* L. var. Gila). Biochim. Biophys. Acta **754**, 292–297

Stymne, S., Appelqvist, L.-Å. (1978) The biosynthesis of linoleate from oleoyl-CoA via oleoyl-phosphatidylcholine in microsomes of developing safflower seeds. Eur. J. Biochem. **90**, 223–229

Stymne, S., Glad, G. (1981) Acyl exchange between oleoyl-CoA and phosphatidylcholine in microsomes of developing soya bean cotyledons and its role in fatty acid desaturation. Lipids **16**, 298–305

Stymne, S., Stobart, A.K. (1984a) The biosynthesis of triacylglycerols in the microsomal preparations of developing cotyledons of sunflower. Biochem. J. **220**, 481–488

Stymne, S., Stobart, A.K. (1984b) Evidence for the reversibility of the acyl-CoA: lysophosphatidylcholine acyltransferase in the microsomes of developing safflower cotyledons and rat liver. Biochem. J. **223**, 305–314

Stymne, S., Stobart, A.K. (1985) Oil synthesis in vitro in microsomal membranes from developing cotyledons of *Linum usitatissimum* L. Planta **164**, 101–104

Stymne, S., Stobart, A.K. (1987) Triacylglycerol biosynthesis. In: The biochemistry of plants, vol. 10, Stumpf, P.K., ed. Academic Press, New York (in press)

Stymne, S., Stobart, A.K., Glad, G. (1983) The role of the acyl-CoA pool in the synthesis of polyunsaturated 18-carbon fatty acids and triacylglycerol production in the microsomes of developing safflower seeds. Biochim. Biophys. Acta **752**, 198–208

Wilson, R.F., Weissinger, H.H., Buck, J.A., Faulkner, J.D. (1980) Involvement of phospholipids in polyunsaturated fatty acid synthesis in developing soyabean cotyledons. Plant Physiol. **66**, 545–549

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