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Δ^6- and Δ^{12}-desaturase activities and phosphatidic acid formation in microsomal preparations from the developing cotyledons of common borage (*Borago officinalis*)

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Microsomal membrane preparations from the maturing cotyledons of common borage (*Borago officinalis*) exhibit Δ^{12}- and Δ^6-desaturase activities, which resulted in the synthesis of linoleate and γ-linolenate respectively. The desaturase enzymes utilized the complex lipid substrate phosphatidylcholine. The activity of these enzymes was sufficiently high to allow the monitoring of the mass changes in the endogenous oleate, linoleate and γ-linolenate in the microsomal phosphatidylcholine in the presence of NADH (i.e. under desaturating conditions). The results illustrate that the Δ^{12}-desaturase uses the oleate substrate at both the *sn*-1 and -2 positions of *sn*-phosphatidylcholine, whereas the Δ^6-desaturase is almost totally restricted to the linoleate at position 2 of the complex lipid. Estimate of the acyl-substrate pool size at position 2 of *sn*-phosphatidylcholine for both desaturases indicated that some 50 % of the oleate and linoleate was available to the enzymes. The microsomes (microsomal fractions) had a somewhat impaired Kennedy [(1961) Fed. Proc. Fed. Am. Soc. Exp. Biol. **20**, 934–940] pathway for the formation of triacylglycerols when compared with other oil-rich plant species that have been studied [Stymne & Stobart (1987) The Biochemistry of Plants: a Comprehensive Treatise (Stumpf, P. K., ed.), vol. 10, chapter 8, pp. 175–214, Academic Press, New York]. In the presence of *sn*-glycerol 3-phosphate and acyl-CoA, large quantities of phosphatidic acid accumulated in the membranes. Acyl-selectivity studies on the glycerol-acylating enzymes showed that γ-linolenate could be acylated to both the *sn*-1 and *sn*-2 positions of *sn*-glycerol 3-phosphate. However, stereochemical analysis of the acyl components of the *sn*-triacylglycerol obtained from mature seeds indicated that, whereas no γ-linolenate was present at the *sn*-1 position, it accounted for over 50 % of the fatty acids at position *sn*-3. The results indicate that the diacylglycerol acyltransferase (EC 2.3.1.20) may show a strong selectivity for γ-linolenoyl-CoA and hence result in the efficient removal of this fatty acid from the acyl-CoA pool *in vivo*, leaving negligible substrate for utilization by the *sn*-glycerol 3-phosphate acyltransferase (EC 2.3.1.15).

INTRODUCTION

Members of the plant family Boraginaceae are characterized by the presence of the uncommon fatty acid γ-linolenic acid (octadeca-6,9,12-trienoic acid) (Kleiman *et al.*, 1964; Jamieson & Reid, 1968, 1969). The triacylglycerol seed oils of the common borage (*Borago officinalis*) are particularly enriched with this fatty acid (Tetenyi *et al.*, 1974). Previous observations showed (Stymne & Stobart, 1986) that the microsomal-membrane preparations from the maturing cotyledons of borage harvested at an early stage in oil deposition contained Δ^{12}- and Δ^6-desaturase enzymes that catalysed the conversion of oleate (octadeca-9-enoic acid) into linoleate (octadeca-9,12-dienoic acid) and finally into γ-linolenate. The results also indicated that the Δ^6-desaturase utilized linoleoyl phosphatidylcholine as the substrate and that the enzyme may be specific for the acyl substrate at position 2 of the complex lipid. On the other hand, the Δ^{12}-desaturase enzyme(s) in maturing seeds of safflower (*Carthamus tinctorius*) that convert oleate into linoleate do so with the substrate present in both positions 1 and 2 of microsomal *sn*-phosphatidylcholine (Slack *et al.*, 1979; Stobart & Stymne, 1985). Previous studies *in vivo* and *in vitro* with the developing seeds of linoleate-rich species have shown that the quality of the fatty acids in the acyl-CoA pool is controlled by the activity of an acyl-CoA:lysophosphatidylcholine acyltransferase (EC 2.3.1.23) (Stymne & Stobart, 1987). The acyltransferase may be responsible for the entry of oleate into position 2 of *sn*-phosphatidylcholine and the return of polyunsaturated products to the acyl-CoA pool, from which they can be used in the acylation of *sn*-glycerol 3-phosphate to yield phosphatidic acid and, finally, triacylglycerol (Stymne & Stobart, 1987). The present investigation continues our studies on the Δ^6-desaturase in *Borago* and, in particular, the confirmation of the positional specificity of the enzyme. We also present further observations of the acyl-substrate-specificity properties of the desaturase enzymes and the utilization of the γ-linolenate in the acylation of *sn*-glycerol 3-phosphate.

MATERIALS AND METHODS

Chemicals

[1-^{14}C]Stearic acid (octadecanoic acid), [1-^{14}C]oleic acid (octadeca-9-enoic acid) and L-*sn*-[U-^{14}C]glycerol 3-phosphate were obtained from The Radiochemical

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Centre, Amersham, Bucks., U.K. Catalase (thymol-free, 11000 units/mg), bovine serum albumin (fraction V, fatty-acid-free), CoASH, NADH, phospholipase A_2 [from Indian-cobra (*Naja naja*) venom], L-*sn*-glycerol 3-phosphate and various fatty acids were purchased from Sigma.

Palmitoyl-CoA (hexadecanoyl-CoA), [1-^{14}C]oleoyl-CoA (sp. radioactivity 12746 d.p.m./nmol), oleoyl-CoA, linoleoyl-CoA (octadeca-9,12-dienoyl-CoA) and γ-linolenoyl-CoA (octadeca-6,9,12-trienoyl-CoA) were synthesized from their mixed anhydrides as described by Sanchez *et al.* (1973). The radioactive acyl-CoA species had a purity greater than 97% as determined by t.l.c., g.l.c. and radio-g.l.c. L-*sn*-[U-^{14}C]Glycerol 3-phosphate was diluted with non-radioactive *sn*-glycerol 3-phosphate to give a specific radioactivity of 1000 d.p.m./nmol.

Plant material and microsomal preparations

Borago officinalis L. (common borage) plants were grown from seeds (generously provided by Dr. Ulf Nyman, Hilleshög Ltd., Landskrona, Sweden) in a 16 h photoperiod at 18 °C and an 8 h night at 13 °C. The flowers were hand-pollinated, and the developing seeds were harvested 15–20 days after flowering.

Cotyledons (approx. 5 mg fresh wt./cotyledon pair) were removed from the developing seeds and stored in ice-cold 0.1 M-potassium phosphate buffer, pH 7.2. All further procedures were carried out at 1–4 °C. The cotyledons (1 part) were ground in a mortar with two parts (w/v) of 0.1 M-potassium phosphate buffer, pH 7.2, containing 0.1% bovine serum albumin, 0.33 M-sucrose and 1000 units of catalase/ml. The homogenate was diluted 20-fold with fresh grinding medium and filtered through a double layer of Miracloth before centrifugation at 20000 *g* for 10 min. The supernatant was re-filtered through Miracloth and centrifuged at 105000 *g* for 90 min. The resulting microsomal pellet was resuspended in the homogenization medium and used immediately.

Enzyme assays

Microsomal assays were carried out at 25 °C with constant shaking and in complete darkness. The incubation mixtures contained 10 mg of bovine serum albumin, 1000 units of catalase, 66 mM-sucrose, substrates and cofactors (as given in the Tables and Figures) and microsomal membranes in a final volume of 1 ml with 0.1 M-phosphate buffer.

Analytical procedures

Reactions were terminated by the addition of 0.15 M-acetic acid, and the lipids were extracted in a modification of the medium described by Bligh & Dyer (1959). The lower chloroform phase, which contains the complex lipids and the non-esterified fatty acids, was removed and evaporated to dryness under N_2. The residue was dissolved in a small volume of chloroform, and the polar and neutral lipids were purified by t.l.c. on precoated silica-gel plates (Merck; silica-gel 60) with chloroform/methanol/acetic acid/water (170:30:20:7, by vol.) or hexane/diethyl ether/acetic acid (70:30:1, by vol.) respectively. Lipid areas, located by lightly staining with I_2 vapour, were removed from the plates and either assayed for radioactivity or methylated *in situ* with methanolic 2.5% (w/w) HCl (Kates, 1964) for the analysis of the fatty acids. The fatty acid methyl esters were analysed by g.l.c. with a glass column (2 m × 2 mm) containing 10% BDS on Chromosorb W (HP, 80–100 mesh) and quantified by using methylheptadecanoic acid as an internal standard. Radioactive fatty acid methyl esters were analysed by radio-g.l.c., and specific radioactivities were determined with methyl [1-^{14}C]-stearate of known specific radioactivity (2045 d.p.m./nmol). Methyl esters of γ-linolenic acid were authenticated as previously described (Stymne & Stobart, 1986).

Positional analysis of the fatty acids in the phospholipids was performed by treatment of the lipid with phospholipase A_2 as previously described (Griffiths *et al.*, 1985). Stereochemical analysis of the fatty acids in the triacylglycerol was carried out essentially as described by Christie (1982). Triacylglycerols isolated from mature seeds were hydrolysed with ethyl magnesium bromide, and the resulting mixture of 1,2- and 2,3-diacylglycerols purified at 4 °C on boric acid-impregnated t.l.c. plates [Merck silica-gel 60 treated with 5% (w/w) boric acid] with hexane/diethyl ether (1:1, v/v). The purified species of diacylglycerols were converted into phosphatidylcholine (Myher & Kuksis, 1979). The *sn*-1,2-diacyl-3-phosphocholine was then hydrolysed using phospholipase A_2 and the resulting fatty acids and lyso derivatives purified, transmethylated and quantified as described above. The distribution of fatty acids at position 3 of the *sn*-triacylglycerols was deduced from the acyl composition of the total triacylglycerols and those present at positions *sn*-1 and *sn*-2.

Lipid samples were assayed for radioactivity in PCS (Amersham/Searle)/xylene (2:1, v/v) scintillant in a Beckman LS-230 liquid-scintillation counter with an efficiency of 94% for ^{14}C. All radioactivity counts were corrected for background and quenching.

RESULTS AND DISCUSSION

Desaturation of the endogenous oleate and linoleate in the microsomal lipids of borage cotyledons

Microsomal preparations of developing borage cotyledons, when incubated in the presence of NADH, readily desaturated the endogenous oleate and linoleate in the phosphatidylcholine. A substantial decrease in the oleate and an increase in the γ-linolenate of the phosphatidylcholine was observed (Table 1). In incubations without the addition of NADH, no significant change occurred in the fatty acid composition of the complex lipids (Table 1). The linoleate and γ-linolenate were also found to increase, to some extent, in the microsomal phosphatidylethanolamine (Table 1). Whether the changes in the fatty acids in phosphatidylethanolamine are due to desaturase activity or the involvement of transacylation reactions (Trotter & Ferber, 1981) remains to be determined. It is noteworthy, however, that there is negligible contribution of fatty acids in phosphatidylethanolamine for triacylglycerol formation *in vivo* and *in vitro* for the many oil-seed species that have been studied (Stymne & Stobart, 1987).

A time-course study of the desaturation of the endogenous oleate and linoleate at the *sn*-1 and *sn*-2 positions of *sn*-phosphatidylcholine, during an incubation of microsomal membrane preparations with NADH, is presented in Fig. 1. At position *sn*-1 (Fig. 1*a*), the oleate content rapidly decreased and, in the first

Table 1. Fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in microsomal preparations incubated in the absence or presence of NADH

Microsomal preparations from the developing cotyledons of borage (equivalent to 110 nmol of phosphatidylcholine) were incubated in the absence or presence of NADH (2 μmol) at 25 °C at the times stated in the Table. For incubation conditions and analytical procedures, see the Materials and methods section. The results shown are from a representative experiment.

Lipid	Incubation time (min)	NADH	Fatty acid...	Fatty acid distribution (%)*				
				$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{\gamma\text{-}18:3}$
Phosphatidylcholine	0	−		17	6	28	36	13
	320	−		16	6	29	37	13
	20	+		17	6	16	45	16
	40	+		17	6	12	45	19
	80	+		16	6	11	44	23
	160	+		16	6	11	41	26
	320	+		17	6	10	39	28
Phosphatidylethanolamine	0	−		23	3	11	54	9
	320	+		23	4	5	54	14

* Small amounts of $C_{20:1}$ fatty acid (< 2%) are not included in the calculation.

40 min, declined from about 24 to 11% of the total fatty acids present, and there was a concomitant increase in the linoleate from 30 to 43%. In incubations longer than 40 min, only slight changes were observed in the content of oleate and linoleate. The γ-linolenate in position *sn*-1 of *sn*-phosphatidylcholine was originally very low (1.4%), but this increased to about 4% after a prolonged incubation of 320 min with NADH.

At position *sn*-2 of phosphatidylcholine there was, similarly to position *sn*-1, a rapid decline in the oleate content during the first 40 min of incubation (from 38 to 17%; Fig. 1*b*), after which the level remained almost the same for the remaining period of incubation. During the first 20 min, under desaturating conditions, both the linoleate and γ-linolenate content rose from 42 to 53% and from 21 to 27% respectively. After 20 min of incubation there was a continuous decrease in the linoleate content from 53 to 36% and an increase in the γ-linolenate from 27 to 49%. No significant change in the total phosphatidylcholine was observed during the first 40 min of incubation, whereas, at prolonged incubation times (320 min), there was a substantial decrease (38%) in this lipid (results not shown). The synthesis of γ-linolenate was rapid and linear during the first 40 min of incubation (0.3 nmol/min per 100 nmol of phosphatidylcholine), after which the desaturation rate slowed down considerably (0.08 nmol/min per 100 nmol of phosphatidylcholine) between 40 and 60 min.

In previous work with *Borago* microsomes (Stymne & Stobart, 1986), it was noted that there was an inverse relationship between the desaturation of oleate and the appearance of γ-linolenate in phosphatidylcholine, whereas little change was observed in the linoleate content. In the present study there were also only relatively small changes, at prolonged incubation times, in the linoleate content at position *sn*-2 of *sn*-phosphatidylcholine during the sequential desaturation of oleate to γ-linolenate (Fig. 1*b*). It has also been noted that the fatty acid composition of the seed oils from a large number of different borage populations exhibit an inverse relationship between the oleate and γ-linolenate content and no correlation between the oleate content and that of linoleate (U. Nyman, personal communication). These observations evoke the question of whether there is a direct channelling (see Murphy *et al.*, 1984) of the linoleate produced by the Δ^{12}-desaturase to the site of the Δ^{6}-desaturase. This would necessitate that the bulk pool of linoleate in position *sn*-2 of *sn*-phosphatidylcholine is not available to the Δ^{6}-desaturase. In order to test this possibility, *Borago* microsomal *sn*-phosphatidylcholine was prelabelled with [^{14}C]oleate at position *sn*-2, by incubation of the microsomal membranes with [^{14}C]-oleoyl-CoA in the presence of bovine serum albumin and free CoA and under non-desaturating conditions (minus NADH). The conditions of incubation were chosen to promote the acyl exchange between the acyl-CoA and position 2 of *sn*-phosphatidylcholine (Stymne & Stobart, 1984). The transfer of the [^{14}C]oleate, in acyl-CoA, to phosphatidylcholine was terminated after 20 min by diluting the incubation mixture with cold homogenization buffer. The microsomal membranes were recovered by a further centrifugation. Some 75% of the radioactivity in the pretreated and washed microsomes resided in position *sn*-2 of *sn*-phosphatidylcholine (results not shown). Portions of the [^{14}C]oleate-labelled microsomes (equivalent to 95 nmol of phosphatidylcholine) were further incubated for 60 min with NADH. The mass and the specific radioactivities of the [^{14}C]oleate, [^{14}C]linoleate and the γ-[^{14}C]linolenate in position *sn*-2 of *sn*-phosphatidylcholine were determined before and after desaturation. The results (Table 2) show that the specific radioactivity of the [^{14}C]oleate decreased some 40% during the incubation with NADH. Thus the oleate derived from [^{14}C]oleoyl-CoA (which represented about 30% of the total oleate now present in position *sn*-2 of *sn*-phosphatidylcholine) was desaturated to a substantially greater extent than was the bulk pool of oleate at position *sn*-2. The oleate that had undergone desaturation (14 nmol) would therefore have had a specific radioactivity of about 66.5×10^2 d.p.m./nmol. If the γ-linolenate that was produced (5.2 nmol) was synthesized via a pool of linoleate that was distinct from the bulk pool at position *sn*-2, then the specific radioactivity of the γ-linolenate at 60 min of incubation

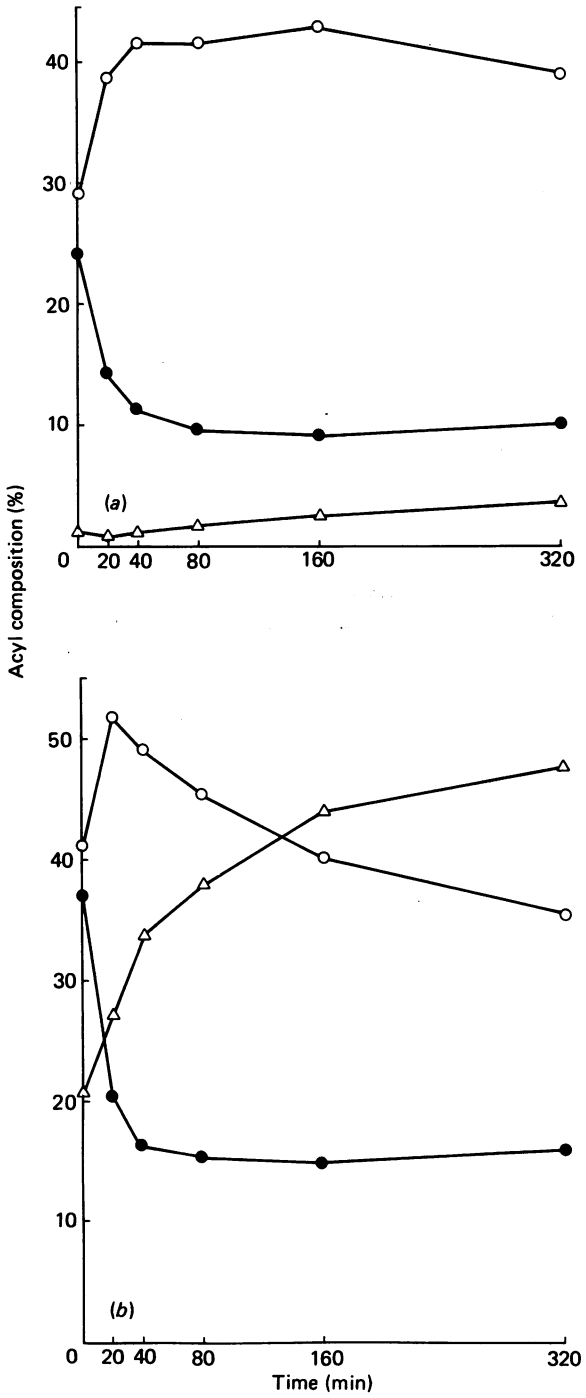

Fig. 1. Desaturation of oleate and linoleate at positions *sn*-1 (*a*) and *sn*-2 (*b*) of the *sn*-phosphatidylcholine in microsomal preparations from the developing cotyledons of borage

Microsomal preparations from the developing cotyledons of borage (equivalent to 110 nmol of phosphatidylcholine) were incubated with NADH (2 μmol) at 25 °C. At regular intervals the incubations were terminated, lipids extracted and phosphatidylcholine isolated and treated with phospholipase A_2. The resulting lysophosphatidylcholine (position *sn*-1, *a*) and free fatty acids (position *sn*-2, *b*) were purified, transmethylated and analysed by g.l.c. The results for the saturated fatty acids (only present in position *sn*-1) are not given. The data are for single determinations only. The experiment, however, was repeated with a different microsomal preparation and with similar results. Symbols used: ●, oleate; ○, linoleate; △, γ-linolenate.

Table 2. Desaturation of *sn*-2-[^{14}C]oleoyl phosphatidylcholine labelled *in situ* in microsomal preparations from the developing cotyledons of borage

Microsomal preparations (equivalent to 1.1 μmol of phosphatidylcholine) were prelabelled with [^{14}C]oleate by incubation for 20 min at 25 °C with 225 nmol of [^{14}C]oleoyl-CoA (sp. radioactivity 12746 d.p.m./nmol), 600 nmol of CoA, 30 mg of bovine serum albumin, 3000 units of catalase in 0.1 M-phosphate buffer, pH 7.2, in a total volume of 3 ml. The reaction mixture was then diluted to 25 ml with cold homogenization buffer and the microsomes collected by centrifugation at 105000 *g* for 60 min. After resuspension of the microsomes, portions (equivalent to 95 nmol of phosphatidylcholine) were either extracted immediately (zero-time samples) or incubated with NADH (2 μmol) for 60 min. Results are the means ± S.E.M. for two incubations.

Acyl group at position *sn*-2 of *sn*-phosphatidylcholine ...	Oleate			Linoleate			γ-Linolenate		
Incubation time (min)	^{14}C* (nmol)	Mass (nmol)	$10^{-2}\times$ Sp. radioactivity (d.p.m./nmol)	^{14}C* (nmol)	Mass (nmol)	$10^{-2}\times$ Sp. radioactivity (d.p.m./nmol)	^{14}C* (nmol)	Mass (nmol)	$10^{-2}\times$ Sp. radioactivity (d.p.m./nmol)
0	10.5±0.6	30.4±0.5	46.3±0.3	0	39.2±0.2	0	0	24.8±0.2	0
60	3.4±0.1	17.0±0.5	25.8±0.3	4.3±0.1	48.5±0.6	11.3±0.4	0.8±0.1	30.0±0.1	3.5±0.4

* nmol of ^{14}C is calculated on the basis of the original sp. radioactivity of the [^{14}C]oleoyl-CoA substrate.

would have been about 11.5×10^2 d.p.m./nmol. The observed value, however, was 3.5×10^2 d.p.m./nmol. Since most of the oleate is desaturated within the first 20 min of incubation, when only a small amount of γ-linolenate had been produced (see Fig. 1*b*), it has been assumed, to facilitate further interpretation, that all the oleate was desaturated before that of the linoleate. With this assumption, if *all* the linoleate in position *sn*-2 of phosphatidylcholine was available for desaturation, then the γ-linolenic acid would have had a specific radioactivity of 3.0×10^2 d.p.m./nmol, and this is close to the observed value (see Table 2). However, since some of the γ-linolenate is in reality produced during active oleate desaturation (Fig. 1*b*), then the assumptions made in the calculation will clearly overestimate the specific radioactivity of the γ-linolenate produced (i.e. a substantial part of the γ-linolenate will, at early incubation times, be synthesized from a linoleate pool of much lower specific radioactivity). The observed specific radioactivity (350 d.p.m./nmol) for the γ-linolenate after 60 min of incubation indicates, therefore, that the [^{14}C]linoleate, which is produced from [^{14}C]oleate at position *sn*-2 of *sn*-phosphatidylcholine, is diluted by a substantial part (but not all) of the endogenous linoleate before further desaturation to γ-linolenate has occurred. It can be estimated from these data that at least half of the oleate and linoleate substrates in position *sn*-2 of *sn*-phosphatidylcholine is available for the synthesis of γ-linolenate. However, the decrease in the specific radioactivity of the [^{14}C]oleate in phosphatidylcholine during desaturation (Table 2) may indicate that some of the newly acylated species are somewhat more readily utilized in linoleate formation than is the bulk pool of *sn*-2-oleoyl-phosphatidylcholine (see Murphy *et al.*, 1984). Nonetheless, the results strongly indicate that there is little direct channelling of the oleate to γ-linolenate through a small active pool of linoleate.

Stereospecific analysis of the fatty acids in the triacylglycerols of *Borago* cotyledons

In order to establish the possible pathway(s) of γ-linolenate incorporation into the triacylglycerols, it is essential to know its stereospecific distribution in this lipid. The results of such an analysis are presented in Table 3. γ-Linolenate was present in only trace quantities at position *sn*-1, whereas it amounted to 44 and 53% of the fatty acids at positions *sn*-2 and *sn*-3 respectively. Palmitate and stearate were absent in position *sn*-2 and enriched in position *sn*-1. Substantial quantities of oleate and linoleate were present in all positions.

Utilization of γ-linolenoyl-CoA in the acylation of *sn*-glycerol 3-phosphate in microsomal preparations

The utilization of γ-linolenate by the reactions of the so-called 'Kennedy (1961) Pathway' was investigated by incubating microsomal membranes with mixtures of γ-linolenoyl-CoA and other acyl-CoA species in the presence of *sn*-[^{14}C]glycerol 3-phosphate. The experiments had to be carried out with non-radioactive acyl-CoA species, since there was no commercially available radioactive γ-linolenic acid. The results (Table 4) show that the microsomes contained active *sn*-glycerol 3-phosphate-acylating enzymes, which yielded phosphatidic acid (Table 4). There was, however, little further metabolism of the phosphatidic acid compared with previous findings with microsomal preparations from developing cotyledons of other oilseeds (Stymne & Stobart, 1987). It should be noted that the experiments reported in Table 4 were carried out in the presence of Mg^{2+}, a bivalent cation that has been found to stimulate the activity of the phosphatidate phosphohydrolase in other oilseed preparations (Griffiths *et al.*, 1985). The results also show (Table 4) that, when γ-linolenoyl-CoA is presented to the microsomes together with an equimolar amount of palmitoyl-CoA, then the total amount of phosphatidic acid produced is only about 40% of that found in incubations with γ-linolenoyl-CoA in combination with either oleoyl-CoA, linoleoyl-CoA or a mixture of equimolar amounts of palmitoyl-, oleoyl-, linoleoyl- and γ-linolenoyl-CoA. Some 20–30% of the ^{14}C incorporated from *sn*-[^{14}C]glycerol 3-phosphate was found in complex lipids other than phosphatidic acid. Of this radioactivity, the major part resided in phosphatidylcholine, with only trace amounts in the diacylglycerol and triacylglycerol (Table 4). Compared with membrane preparations from the developing seeds of other species (Stymne & Stobart, 1987) the microsomes appeared to have relatively low phosphohydrolase activity. Whether the phosphohydrolase was lost from the membranes during preparation remains to be ascertained. However, we have noted in recent experiments that freshly prepared homogenates from borage were capable of efficient triacylglycerol production from *sn*-glycerol 3-phosphate and acyl-CoA (G. Griffiths, A. K. Stobart & S. Stymne, unpublished work).

The fatty acid composition of the total phosphatidic acid synthesized during incubation of the microsomes with acyl-CoA and *sn*[^{14}C]glycerol 3-phosphate and under non-desaturating conditions was determined (Table 5). It is noteworthy that, in incubations with γ-linolenoyl-CoA and palmitoyl- or oleoyl-CoA, some linoleate had been utilized in the acylation of *sn*-glycerol 3-phosphate (Table 5). The linoleate was, most probably, derived from position *sn*-2 of *sn*-phosphatidylcholine via acyl exchange with unsaturated acyl-CoA species (Griffiths *et al.*, 1985). γ-Linolenoyl-CoA was acylated to *sn*-glycerol 3-phosphate at a lower rate in the presence of palmitoyl-CoA compared with incubations in which the γ-linolenate had to compete with the unsaturated acyl-CoA species (Table 5).

Table 3. Positional distribution of acyl groups in the triacylglycerols in the cotyledons of borage seeds

Triacylglycerols from cotyledons of mature borage seeds were purified and a positional analysis of the constituent fatty acids was carried out (see the Materials and methods section).

Position	Fatty acid...	Fatty acid distribution (%)					
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{\gamma\text{-}18:3}$	$C_{20:1}$
sn-1		23	6	16	51	0	4
sn-2		0	0	17	39	44	0
sn-3*		5	0	7	31	53	4
Total triacylglycerol		9	2	14	40	32	3

* Deduced from the fatty acid distribution found in positions *sn*-1 and *sn*-2 and in the total triacylglycerols.

Table 4. The incorporation of [^{14}C]glycerol from *sn*-[^{14}C]glycerol 3-phosphate into lipids in the presence of a mixture of unlabelled acyl-CoA in incubations of microsomal preparations of developing cotyledons of borage

Microsomes (equivalent to 64 nmol of phosphatidylcholine) were incubated with *sn*-[^{14}C]glycerol 3-phosphate (800 nmol) and acyl-CoA species (at concentrations stated in the Table) in the presence of bovine serum albumin (20 mg) and $MgCl_2$ (10 μmol) in 0.1 M-phosphate buffer, pH 7.2, in a total volume of 1 ml. Incubations were allowed to proceed for 60 min at 25 °C. For experimental details, see the Materials and methods section. The results are means (±S.E.M.) for two incubations.

		[^{14}C]Glycerol incorporated (nmol)					
Acyl-CoA substrate	Lipid...	Phosphatidyl-choline	Phosphatidic acid	Diacyl-glycerol	Triacyl-glycerol	Other lipids	Total lipid
$C_{16:0}$-CoA + $C_{\gamma\text{-}18:3}$-CoA (250 nmol each)		3.2±0.2	15.8±0.8	0.1±0	0.1±0	2.9±0.1	22.2±1.2
$C_{18:1}$-CoA + $C_{\gamma\text{-}18:3}$-CoA (250 nmol each)		5.9±0.3	41.0±2.0	0.6±0	0.9±0	2.6±0.1	50.9±2.6
$C_{18:2}$-CoA + $C_{\gamma\text{-}18:3}$-CoA (250 nmol each)		5.6±0.3	37.2±1.1	0.7±0	1.0±0	5.7±0.1	47.3±1.2
$C_{16:0}$-CoA + $C_{18:1}$-CoA + $C_{18:2}$-CoA + $C_{\gamma\text{-}18:3}$-CoA (125 nmol each)		6.7±1.3	41.0±7.9	0.8±0.1	1.2±0.2	5.2±1.0	54.9±10.6

Table 5. Fatty acid composition of the phosphatidate synthesized in microsomes from developing borage cotyledons incubated with *sn*-glycerol 3-phosphate and mixtures of acyl-CoA species

Incubation conditions were identical with those described in Table 4. The phosphatidate was isolated and transmethylated. The results shown are single determinations of two pooled incubations.

		Phosphatidate composition (nmol)*				
Acyl-CoA substrate	Fatty acid...	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{\gamma\text{-}18:3}$
$C_{16:0}$-CoA + $C_{\gamma\text{-}18:3}$-CoA		21	0	1	8	19
$C_{18:1}$-CoA + $C_{\gamma\text{-}18:3}$-CoA		1	0	88	11	43
$C_{18:2}$-CoA + $C_{\gamma\text{-}18:3}$-CoA		0	0	1	80	38
$C_{16:0}$-CoA + $C_{18:1}$-CoA + $C_{18:2}$-CoA + $C_{\gamma\text{-}18:3}$-CoA		31	0	6	52	22

* Fatty acids of phosphatidate are corrected for background, i.e. 'zero' time.

The positional distribution of γ-linolenic acid in the synthesized phosphatidic acid revealed that this acid had been utilized in the acylation of both the *sn*-1 and *sn*-2 positions of the *sn*-glycerol 3-phosphate (results not shown). The acyl selectivity observed for the *sn*-glycerol 3-phosphate-acylating enzymes is rather unexpected in the light of the intramolecular distribution of γ-linolenate in the triacylglycerols (Table 3). If it is assumed that a common pool exists for all the species of acyl-CoA that are available for the acylation of the glycerol backbone, then the results may indicate that the enzyme diacylglycerol acyltransferase (EC 2.3.1.20) is somewhat selective for the γ-linolenate substrate. Such a selectivity may, therefore, efficiently scavenge γ-linolenoyl-CoA [derived from position *sn*-2 of *sn*-phosphatidylcholine via acyl exchange (Stymne & Stobart, 1984)] and thus effectively limits its acylation to position *sn*-1 of *sn*-glycerol 3-phosphate. Some acyl selectivity had been reported for the diacylglycerol acyltransferase in maize (*Zea mays*) (Cao & Huang, 1986), but this was not so apparent in safflower preparations (Ichihara *et al.*, 1987). We propose that, in borage and perhaps other species, the diacylglycerol acyltransferase can not only regulate the acyl quality at position *sn*-3 of the *sn*-triacylglycerol, but can also modulate the fatty acid composition at the other positions in the triacylglycerol molecule.

In conclusion, the experiments reported above confirm, beyond doubt, that the Δ^6-desaturase enzyme is restricted in its substrate almost totally to the linoleate that is located at the *sn*-2 position of *sn*-phosphatidylcholine and exhibits little activity with the substrate at the *sn*-1 position. The Δ^{12}-desaturase, on the other hand, utilized very efficiently the oleate at both the *sn*-1 and *sn*-2 positions in the complex lipid. Previous studies with oilseeds of other species also show that the formation of linoleate from oleate (i.e. Δ^{12}-desaturase activity) will occur in the *sn*-1 and -2 positions of *sn*-phosphatidylcholine. These observations raise interesting questions regarding the desaturase enzymes in general that utilize acyl substrates esterified to complex lipids. For instance, is the Δ^{12}-desaturase activity that is observed at positions *sn*-1 and -2 of *sn*-phosphatidylcholine catalysed by different enzymes? How is the Δ^6-desaturase enzyme restricted to position *sn*-2 when the enzyme is presumably moving freely in the bilayer of the membrane (ie. floating in its own complex-lipid substrate)? Do other plant

species exist in which the Δ^{12}- and Δ^{15}-desaturase enzymes also show positional specificity? Answers to questions such as these are not only important for a fundamental understanding of desaturase action in plants, but have wider implications in discerning the strategies required for regulating acyl quality through plant breeding and genetic engineering.

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