Evidence for an Oleoyl Phosphatidylcholine Desaturase in Microsomal Preparations from Cotyledons of Safflower (*Carthamus tinctorius*) Seed

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1. [^{14}C]Oleoyl-CoA was metabolized rapidly and essentially completely by microsomal preparations from developing safflower (*Carthamus tinctorius*) cotyledons, and most of the [^{14}C]oleate was incorporated into 3-*sn*-phosphatidylcholine. 2. In aerobic reaction mixtures containing NADH₂ the [^{14}C]oleate in 3-*sn*-phosphatidylcholine was converted into [^{14}C]linoleate without any change in the specific radioactivity of the lipid. Over a 60 min incubation period the extent of conversion of [^{14}C]oleoyl phosphatidylcholine into [^{14}C]linoleoyl phosphatidylcholine was generally greater than 60%. The rate of desaturation of endogenous [^{14}C]oleoyl phosphatidylcholine labelled from [^{14}C]oleoyl-CoA was much greater than that of exogenous [^{14}C]dioleoyl phosphatidylcholine. 3. During the initial period of desaturation of endogenous 3-*sn*-phosphatidylcholine the specific radioactivity of the oleoyl moiety of the lipid remained constant, indicating that labelled and unlabelled oleate were desaturated at the same rate. On this assumption an initial rate of desaturation of about 15 nmol of oleate desaturated/min per μmol of 3-*sn*-phosphatidylcholine was estimated. 4. [^{14}C]Oleate esterified at positions 1 and 2 of both endogenous and exogenous 3-*sn*-phosphatidylcholine was desaturated. 5. Attempts to demonstrate the presence of an oleoyl-CoA desaturase in safflower microsomal fractions by the appearance of linoleoyl-CoA in reaction mixtures were inconclusive.

Labelling studies of the metabolism of C₁₈ fatty acids in detached developing cotyledons of linseed (*Linum usitatissimum*) (Dybing & Craig, 1970; Slack *et al.*, 1978), safflower (*Carthamus tinctorius*) and soya bean (*Glycine max*) (Slack *et al.*, 1978) were interpreted as indicating that the phospholipid 3-*sn*-phosphatidylcholine is intimately involved in the desaturation of oleate to linoleate. This belief is supported indirectly by the observation that fatty acids of this phospholipid have a high rate of turnover in developing cotyledons *in vivo* (Slack & Roughan 1978). From the kinetics of labelling of C₁₈ fatty acids from [^{14}C]acetate by suspension cultures of soya-bean callus, Wilson *et al.* (1978) also concluded that oleoyl phosphatidylcholine was converted into linoleoyl phosphatidylcholine. They suggested that this could occur either by the direct desaturation of oleate esterified to the phospholipid or by acyl exchange between the phospholipid and CoA and the desaturation of oleoyl-CoA. Stumpf & Porra (1976) and Stumpf & Weber (1977) on the other hand have proposed from labelling studies with developing

soya-bean cotyledons and soya-bean callus suspensions, respectively, that the desaturation of oleate occurs via oleoyl-CoA, and Vijay & Stumpf (1971) concluded from studies of oleate desaturation by microsomal preparations from safflower cotyledons that oleoyl-CoA, not oleoyl phosphatidylcholine, was the substrate for oleate desaturation. In view of these contradictory conclusions, we have re-examined the process of oleate desaturation by safflower microsomal preparations.

Recently Stymne & Appelqvist (1978) have found that oleate from [^{14}C]oleoyl-CoA was esterified to 3-*sn*-phosphatidylcholine of safflower microsomal preparations, then desaturated. No [^{14}C]linoleoyl-CoA was detected in reaction mixtures in which oleate desaturation occurred.

Experimental

Materials

Plants of safflower (*Carthamus tinctorius*, cultivar 0-22) from seed supplied by Crop Research Division, D.S.I.R., Palmerston North, New Zealand, and cultivars VFSTP-1 and partial hull, high-oleate from seed supplied by Dr. A. L. Urie, Department of

Abbreviations used: Mes, 4-morpholine-ethanesulphonic acid; Hepes, 4-(2-hydroxyethyl)-piperazine-ethanesulphonic acid.

Agronomy and Range Science, University of California, Davis, CA 95616, U.S.A., were grown either in the field during summer or in a controlled environment at a temperature of 22°C day, 18°C night and with a photoperiod of 15 h.

Sodium methoxide was prepared as described previously (Slack *et al.*, 1976) and diazomethane by the method of Schlenk & Gellerman (1960). *sn*-Glycero-3-phosphocholine (CdCl₂ complex) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and CdCl₂ removed from solution by the addition of excess Ag₂CO₃. Neither Cd²⁺ nor Cl⁻ ions in the solution could be detected by the formation of precipitates when samples were added to solutions of K₂CO₃ or AgNO₃ respectively. [¹⁴C]-Oleoyl-CoA (6.5 μCi/μmol) was prepared from [1-¹⁴C]oleic acid (60 μCi/μmol) (The Radiochemical Centre, Amersham, Bucks., U.K.), oleic acid prepared from chromatographically pure methyl oleate (Nu Check Prep, Elysian, MN, U.S.A.) by saponification, and CoA (lithium salt; from Sigma Chemical Co., St. Louis, MO, U.S.A.) by the method of Sánchez *et al.* (1973) and stored in 50 mM-sodium acetate buffer, pH 4.5 at -20°C. Oleoyl-CoA solutions were calibrated by the method of Barber & Lands (1971) and by the amount of methyl oleate produced by transmethylation with sodium methoxide. Estimates by the two methods differed by less than 10%. [¹⁴C]Dioleoyl phosphatidylcholine was prepared from developing seed cotyledons of partial-hull high-oleate safflower by incubating 2.0 g of cotyledons at 25°C with 4 ml of 50 mM-Mes/KOH buffer, pH 5.0, containing 80 μCi of [1-¹⁴C]sodium acetate (60 μCi/μmol) (The Radiochemical Centre) for 2 h. The cotyledons were steamed, the lipids extracted, and the 3-*sn*-phosphatidylcholine purified by t.l.c. and eluted from silica gel as described previously (Slack *et al.*, 1978). The phospholipid was then subjected to argentation chromatography (Arvidson, 1968), the plate sprayed with 0.2% dichloro-fluorescein and four bands identified under u.v. light. The second most mobile band was eluted from silica gel as described by Arvidson (1968), then re-chromatographed on silica-gel G in chloroform/methanol/15 M-NH₃ (65:25:2, by vol.) and stained lightly with I₂ vapour. The lipid containing silica gel was eluted by the sequential addition of 2.0 ml of 50 mM-acetic acid, 3.3 ml of methanol and 6.6 ml of chloroform, the chloroform layer dried, the lipid redissolved in chloroform and stored at -20°C. Samples of the lipid were transmethylated with sodium methoxide and the fatty acid methyl esters analysed by g.l.c. at 180°C on a 2 m × 3 mm column of 15% ethylene glycol succinate on Chromasorb W. The chromatograph was fitted with an effluent-stream splitter that diverted 70% of the gas stream from the column to a collector outlet. Individual fatty acid methyl esters were collected and the

radioactivity in each determined at 80% efficiency with 0.5% (w/v) *p*-terphenyl in xylene as scintillant fluid. The lipid contained 85% oleic acid and was predominantly dioleoyl phosphatidylcholine. The specific radioactivity of the oleate moiety was 1.3 μCi/μmol.

Methods

Microsomal preparation. Flower heads were removed 2–3 weeks after the first florets had appeared, and were placed on ice. Cotyledons were dissected from individual seeds and those between one- and two-thirds final size were retained. About 4 g of these cotyledons were ground with sand with a mortar and pestle, in 5.0 ml of Tris/HCl, pH 7.5, containing 1 mM-EDTA, 1 mM-EGTA, 1 mM-MgCl₂, 0.1% defatted bovine serum albumin, 10 mM-cysteine and 0.33 M-sorbitol. The homogenate was filtered and the residue re-ground and re-extracted twice. The combined filtrates were made to 30 ml with the same medium and centrifuged at 18000g for 10 min. The supernatant from below the oil layer was centrifuged at 105000g for 1 h and the pellet resuspended with a Ten Broek homogenizer in 50 mM-Hepes/KOH, pH 7.0, containing 5 mM-MgCl₂. The 3-*sn*-phosphatidylcholine content of microsomal preparations was determined as described previously (Slack *et al.*, 1976).

Assay of desaturase. Assays were performed at 25°C in screw-cap tubes (12 cm × 2 cm) shaken at 100 strokes/min. Each reaction mixture (0.4 ml) contained microsomal preparation equivalent to between 0.2 and 0.3 g fresh weight of cotyledon tissue (depending on the experiment), 50 mM-Hepes/KOH, pH 7.0, 5 mM-MgCl₂, 2.5 mM-NADH₂ and either [¹⁴C]oleoyl-CoA or [¹⁴C]dioleoyl phosphatidylcholine at concentrations indicated in the Results section. Reactions were started by addition of the labelled substrate. [¹⁴C]Oleoyl-CoA was added to reaction mixtures at 0°C then these were transferred to 25°C. [¹⁴C]Dioleoyl phosphatidylcholine, as a solution in either ethanol or 5% Triton X-100, was added to vigorously shaken reaction mixtures at 25°C. Reactions were stopped by the addition of 4.0 ml of chloroform/methanol (2:1, v/v), 0.4 ml of water was then added and the tubes shaken. For the zero-time assays chloroform/methanol was added to the reaction mixtures before the labelled substrates.

Analysis of reaction products. The chloroform phase was dried under reduced pressure, redissolved in chloroform and samples counted for radioactivity at 80% efficiency as described above. 10% KOH in methanol (1 ml) was added to the aqueous methanol phase plus interfacial material, the tubes sealed, heated at 80°C for 1 h, then 3.6 M-H₂SO₄ (1 ml) added and the solution extracted with 5.0 ml of light petroleum (b.p. 40–60°C). Samples of this petroleum

phase were counted for radioactivity as described above and, where required, the fatty acids were methylated with diazomethane and methyl esters separated and analysed as described below.

To determine the distribution of radioactivity in chloroform-soluble compounds, samples were chromatographed in chloroform/methanol/15M-NH₃ as described above, lipids stained lightly with iodine and the origin plus phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, fatty acids and neutral-lipid regions transferred individually to 0.5% (w/v) *p*-terphenyl in xylene/Triton X-100/water (6:3:1, by vol.) and counted for radioactivity at 60% efficiency. To measure the radioactivity in the oleate and linoleate moieties of 3-*sn*-phosphatidylcholine the lipid was purified by t.l.c. as described above and transmethylated as described previously (Slack *et al.*, 1976). Samples of the methyl esters were either analysed by g.l.c. as above to determine radioactivity in, and mass of, each ester, or co-chromatographed with 50 µg of each of methyl oleate and methyl linoleate on plates of silica-gel G containing AgNO₃ (Morris, 1966). Plates were sprayed with dichlorofluorescein and the areas containing mono- and di-enoic acids identified under u.v. light transferred to the water-containing scintillant described above. Estimates of the distribution of radioactivity between methyl oleate and methyl linoleate by the two methods agreed closely.

To measure the radioactivity in fatty acids esterified to positions 1 and 2 of 3-*sn*-phosphatidylcholine and the extent of [¹⁴C]oleate desaturation at each position, samples of the lipid, purified by t.l.c. and eluted from silica gel as described above, were incubated with lipase from the fungus *Rhizopus arrhizus* (Boehringer-Mannheim G.m.b.H., Mannheim, Germany). The phospholipid, together with 0.2 µmol of 3-*sn*-phosphatidylcholine purified from cotyledons of mature safflower seeds, was suspended in 0.05 ml of 1% sodium cholate and shaken at 20°C with 2 ml of 50 mM-Tris/HCl, pH 7.5, containing 4 mM-CaCl₂ and 100 µg of lipase for 45 min. Chloroform/methanol (2:1, v/v) (10 ml) was added and lipid extracted into the chloroform phase. This was dried under reduced pressure and lysophosphatidylcholine, unhydrolysed phosphatidylcholine and fatty acids separated by t.l.c. in the chloroform/methanol/NH₃ solvent described above. The lipids were stained lightly with I₂ vapour and the two phospholipids transmethylated with sodium methoxide. Fatty acids were eluted from the silica gel as described above for 3-*sn*-phosphatidylcholine and methylated with diazomethane. Fatty acid methyl esters derived from lysophosphatidylcholine and fatty acids were separated by argentation chromatography and radioactivity in each ester determined as above. Other samples of 3-*sn*-phosphatidylcholine were hydrolysed by phospholipase A₂ by using venom of

the king cobra (*Ophiophagus hannah*) (Sigma) as described by Christie (1973), and the hydrolysis products separated and analysed as described above.

Results

Metabolism of [¹⁴C]oleoyl-CoA by microsomal preparations and oleate desaturation

In agreement with previous observations (Baker & Lynen, 1971), most of the radioactivity present in the [¹⁴C]oleoyl-CoA partitioned into the aqueous-methanol phase in the zero-time assays, to which chloroform/methanol was added before the labelled substrate (Tables 1 and 2). Radioactivity in the chloroform phase was located predominantly in fatty acids and compounds that remained at the origin of t.l.c. plates. On incubating microsomal preparations with a low concentration of [¹⁴C]-oleoyl-CoA (Table 1, Expt. a) the oleoyl-CoA was essentially completely metabolized during the first 6 min and, surprisingly, in view of the relatively low rates of oleate desaturation observed previously with microsomal preparations (Baker & Lynen, 1971; Vijay & Stumpf, 1971; Talamo *et al.*, 1973; Slack *et al.*, 1976), oleate desaturation was rapid. After 6 min, 35% of the radioactivity in 3-*sn*-phosphatidylcholine was in linoleate. No labelled linoleate was detected in either fatty acids or in compounds that remained at the origin of chromatograms, which together with 3-*sn*-phosphatidylcholine contained the bulk of the radioactivity in the chloroform phase. During the subsequent 34 min incubation there was no apparent redistribution of radioactivity between lipids, and the specific radioactivity of the 3-*sn*-phosphatidylcholine remained essentially constant; values of 4.5, 4.8, 4.4 and 5.1 × 10⁻⁵ d.p.m./µmol were obtained by g.l.c. analysis for the phospholipid after 6, 10, 20 and 40 min respectively. Similarly in Expt. b (Table 1) where a higher concentration of [¹⁴C]oleoyl-CoA relative to microsomal material was used, the specific radioactivities of the 3-*sn*-phosphatidylcholine after 15 and 50 min were similar at 8.2 and 8.5 × 10⁻⁵ d.p.m./µmol. However, over these periods there was a very considerable loss of radioactivity from the oleoyl moiety and an increase in radioactivity in the linoleoyl moiety of the phospholipid. The extent of the loss of radioactivity from the oleate of 3-*sn*-phosphatidylcholine was considerably greater than the concomitant decline in the specific radioactivity of this moiety, suggesting that a portion of the unlabelled oleate of the phospholipid had been desaturated at a rate comparable with that of the [¹⁴C]oleate. This view is supported by changes in the fatty acid composition of the phospholipid during the assays. Whereas the proportion of saturated fatty acids in the microsomal 3-*sn*-phosphatidylcholine remained constant, that of oleate declined in Expt. a from 8.6 mol/100 mol at 6 min to

Table 1. *Metabolism of [¹⁴C]oleoyl-CoA by microsomal preparations of safflower cotyledons and oleate desaturations at 'low' oleoyl-CoA concentrations*

Unwashed microsomal preparations were incubated with [¹⁴C]oleoyl-CoA under conditions described in the Experimental section. Assays in Expt. a contained 0.36 μ mol and 23 nmol, and in Expt. b 0.23 μ mol and 38 nmol, of microsomal 3-*sn*-phosphatidylcholine and [¹⁴C]oleoyl-CoA respectively. At the times indicated, reactions were stopped by shaking with chloroform/methanol (2.1, v/v) and radioactivity in chloroform and aqueous-methanol layers determined. The distribution of label amongst individual lipids was determined by t.l.c. analysis. 3-*sn*-Phosphatidylcholine (PC) was isolated from the chloroform layer by t.l.c., transmethylated and the radioactivity in fatty acid methyl esters determined as described in the Experimental section.

Expt.	Incubation time (min)	10 ⁻³ × Radioactivity in aqueous-methanol- and chloroform-soluble compounds (d.p.m./assay)		10 ⁻³ × Radioactivity in PC and proportion of total radioactivity of linoleoyl-PC		10 ⁻⁶ × Specific radioactivity of oleoyl PC (d.p.m./ μ mol)
		Aqueous methanol	Chloroform	PC (d.p.m./assay)	Linoleoyl-PC (%)	
a	0	264.5	60.9	—	—	—
	6	1.8	311.2	182.4	35.4	1.55
	10	1.5	326.6	184.5	46.9	1.53
	20	1.4	326.8	173.6	65.7	0.97
	40	1.4	331.3	181.6	77.1	1.08
b	0	485.0	61.2	—	—	—
	5	168.5	363.1	192.0	20.1	1.78
	10	22.3	552.0	304.2	42.7	2.04
	50	25.0	536.3	289.0	70.1	1.58

Table 2. *Metabolism of oleoyl-CoA by microsomal preparations of safflower cotyledons at 'low' oleoyl-CoA concentrations*

Assays containing unwashed microsomal preparations (0.16 μ mol of 3-*sn*-phosphatidylcholine) and 24 nmol of [¹⁴C]oleoyl-CoA were incubated and analysed as described in Table 1, except that fatty acids obtained from the aqueous-methanol phase by saponification were methylated, and radioactivity in fatty acid methyl esters determined, as in the Experimental section.

Incubation time (min)	10 ⁻³ × Radioactivity in water-soluble acyl esters (d.p.m./assay)		10 ⁻³ × Radioactivity in phosphatidylcholine fatty acids (d.p.m./assay)	
	Oleate	Linoleate	Oleate	Linoleate
0.5	117.2	0.1	44.6	0.8
1	98.1	0.2	57.4	2.1
2	86.1	0.4	67.1	6.2
3	63.2	0.4	69.4	10.7
6	19.8	0.3	106.4	23.3

5.6 mol/100 mol at 40 min, with a corresponding increase in the proportion of linoleate. Similarly in Expt. b (Table 1) the proportion of oleate from 12.4 mol/100 mol at 15 min to 8.1 mol/100 mol at 50 min.

The above results strongly suggested that at least the major route of linoleate formation involved the desaturation of oleate esterified to phosphatidylcholine. It was conceivable, however, that some desaturation of oleoyl-CoA could have occurred before the essentially complete incorporation of acyl moieties from thiol esters into the phospholipid. Consequently, we examined the metabolism of [¹⁴C]oleoyl-CoA over a much shorter time period (Table 2). Water-soluble [¹⁴C]oleoyl-CoA remained in the reaction mixtures throughout the experiment, but there was little indication of any accumulation of

radioactivity in linoleoyl-CoA. The small amounts of radioactivity that did co-chromatograph with methyl linoleate when samples of methyl esters derived from the aqueous-methanol phases were analysed by AgNO₃/silica-gel t.l.c. and g.l.c. were inadequate to distinguish whether this radioactivity represented methyl linoleate or the trailing of radioactive material in the chromatographic procedures used. In contrast, the accumulation of radioactivity in the linoleoyl moiety of phosphatidylcholine was considerable and apparently linear after an initial lag period.

In an alternative approach to examine the possibility that linoleoyl-CoA could be formed from oleoyl-CoA, a higher concentration of oleoyl-CoA was incubated with a microsomal preparation

together with ATP and CoA. The latter were included to facilitate the reconversion of any fatty acids, released from acyl-CoA esters by acylthioester hydrolase activity, back to CoA esters by the action of thio-kinase. Under these conditions there was a continuous loss of radioactivity from the thiol-ester fraction and a concomitant accumulation of label in 3-*sn*-phosphatidylcholine (Table 3). Some radioactivity did accumulate in the linoleoyl moiety of a methanol/water-soluble compound, presumably linoleoyl-CoA. However, the amount of radioactivity in this compound compared with that which accumulated in the linoleoyl moiety of phosphatidylcholine was small.

Comparison of the desaturation of [14 C]oleoyl phosphatidylcholine labelled in situ and exogenous [14 C]oleoyl phosphatidylcholine

Two alternative methods were used by Talamo *et al.* (1973) to study directly the desaturation of the oleoyl moiety of 3-*sn*-phosphatidylcholine by microsomal preparations from the yeast *Torulopsis utilis*. In one, [14 C]oleate was incorporated into the phospholipid from [14 C]oleoyl-CoA under non-desaturating conditions, then desaturation allowed to proceed; in the other, [14 C]oleoyl phosphatidylcholine, suspended in detergent, was added to the reaction mixture. By using both methods it was possible to demonstrate the desaturation of the

Table 3. *Metabolism of oleoyl-CoA by microsomal preparations of safflower cotyledons and oleate desaturation at 'high' oleoyl-CoA concentrations*

Assays containing unwashed microsomal preparation [0.36 μ mol of microsomal 3-*sn*-phosphatidylcholine (PC)], 0.115 μ mol of [14 C]oleoyl-CoA, 1.0 μ mol of ATP and 0.4 μ mol of CoA were incubated as outlined in the Experimental section for the times shown and analysed as described in Table 2.

Incubation time (min)	$10^{-4} \times$ Radioactivity in aqueous-methanol phase (d.p.m./assay)	Proportion of radioactivity in linoleate (%)	$10^{-4} \times$ Radioactivity (d.p.m./assay)		Proportion of radioactivity in linoleate PC (%)
			Chloroform-soluble	PC	
0	145.8	0.1	12.2	—	—
6	109.1	0.2	46.9	15.6	12.9
10	97.4	0.4	63.6	26.1	15.9
20	72.8	1.8	87.2	47.1	29.7
40	37.8	4.8	115.6	59.5	35.5

Table 4. *Desaturation of [14 C]oleoyl phosphatidylcholine labelled in situ and of exogenous [14 C]oleoyl phosphatidylcholine*

Expt. a: a microsomal preparation containing about 3.0 μ mol of 3-*sn*-phosphatidylcholine (PC) was incubated under N_2 in 6.0 ml of 50 mM-Hepes/KOH, pH 7.0, containing 5 mM-MgCl₂ with 0.6 μ mol of [14 C]oleoyl-CoA in the absence of NADH₂ for 15 min. The mixture was then diluted to 30 ml with cold N_2 -saturated Hepes/MgCl₂ and microsomal material pelleted by centrifugation at 0°C. Samples of resuspended microsomal material containing 0.19 μ mol of 3-*sn*-phosphatidylcholine were incubated with and without NADH₂ in air for the times indicated. Expt. b: [14 C]-dioleoyl phosphatidylcholine (30 nmol) was added in 0.02 ml of ethanol to reaction mixtures containing microsomal preparation and 0.2 μ mol of 3-*sn*-phosphatidylcholine, and these were incubated for the times indicated. Details of analysis procedures are given in the Experimental section.

Expt.	Substrate	Incubation time (min)	Proportion of radioactivity in linoleate PC (%)	$10^{-6} \times$ Specific radioactivity of oleate moiety of PC
				(d.p.m./ μ mol)
a	Endogenous PC labelled with [14 C]oleate + NADH ₂	0	2.6	10.3
		5	22.3	10.5
		10	32.3	9.5
		60	55.9	7.6
		60	3.6	10.1
b	Exogenous [14 C]dioleoyl PC	0	2.4	2.2
		10	4.9	1.9
		20	8.2	2.2
		60	22.2	2.4

oleoyl moiety of 3-*sn*-phosphatidylcholine by microsomal preparations from safflower cotyledons (Table 4). In Expt. a, [^{14}C]oleate was incorporated into 3-*sn*-phosphatidylcholine from [^{14}C]oleoyl-CoA without desaturation by performing the initial incubation under N_2 without NADH_2 . The [^{14}C]oleoyl phosphatidylcholine labelled *in situ* was desaturated rapidly and, in agreement with the results discussed above, the extent of conversion of [^{14}C]oleate into [^{14}C]linoleate was much greater than the decrease in the specific radioactivity of oleoyl moiety. During the 60 min incubation period the amount of oleate in the phospholipid fell from 18.6 to 11.0 mol/100 mol. However, we have consistently observed less desaturation with exogenous [^{14}C]oleoyl phosphatidylcholine, supplied either dispersed in 5% Triton X-100, at a final detergent concentration of 0.25%, or in solution in ethanol as in Expt. b (Table 3). In this particular experiment, samples of the microsomal preparation were also incubated with [^{14}C]oleoyl-CoA. After 10 min the specific radioactivity of the oleoyl moiety was 5.4×10^{-7} d.p.m./ μmol , and 46% of the radioactivity in the phospholipid was in the linoleoyl moiety. In contrast, only 2.5% of the [^{14}C]oleate of the supplied phospholipid had been desaturated during the same period. At present we have inadequate information to explain satisfactorily the difference between the rates of desaturation of the oleoyl moieties of exogenous and endogenous 3-*sn*-phosphatidylcholine. This discrepancy was not due to inhibition of the desaturase by ethanol added to the assay with the exogenous phospholipid, since the same final ethanol concentration did not inhibit desaturation of [^{14}C]oleate of endogenous 3-*sn*-phosphatidylcholine. However, we do not know either whether the exo-

genous phospholipid that was added to reaction mixtures dissolved uniformly in the lipid phase of the microsomal membranes, or whether exogenous phosphatidylcholine that did dissolve in the microsomal membrane was as readily available to the desaturase as was the endogenous lipid.

Positional specificity for the desaturation of oleoyl phosphatidylcholine

Up to about 25% of radioactivity incorporated into 3-*sn*-phosphatidylcholine from [^{14}C]oleoyl-CoA was present in position 1 (Table 5), a proportion somewhat greater than that observed previously with plant microsomal preparations (Devor & Mudd, 1971; Vijay & Stumpf, 1971; Shine *et al.*, 1976). It was apparent that desaturation had occurred at both positions, irrespective of the extent to which the [^{14}C]oleate had been desaturated, although the amount of desaturation was always somewhat greater at position 2. Similarly, when [^{14}C]dioleoyl phosphatidylcholine (which contained approximately the same amounts of radioactivity in positions 1 and 2) was supplied as the substrate, desaturation occurred at both positions. The quantities of fatty acid methyl esters recovered from position 1 and from position 2 were approximately equal, and the average of the fatty acid compositions at the two positions was the same as that of the 3-*sn*-phosphatidylcholine remaining after enzymic hydrolysis. We suggest, therefore, that the results were not biased either by a differential recovery of fatty acids from the two positions or by the non-random hydrolysis of the lipid. Where analyses were repeated with snake-venom phospholipase A_2 the results were similar to those obtained by using fungal lipase (Table 5). The average fatty acid compositions of position 1

Table 5. *Positional distribution of [^{14}C]oleate and [^{14}C]linoleate in microsomal 3-*sn*-phosphatidylcholine labelled from [^{14}C]oleoyl-CoA and from [^{14}C]dioleoyl phosphatidylcholine under desaturating conditions*

Samples of 3-*sn*-phosphatidylcholine from assays described above were purified by t.l.c. All samples were incubated with fungal lipase and some also with phospholipase A_2 (data in parentheses). Lipid products were separated by t.l.c., and radioactivity in the fatty acid methyl esters prepared from lysophosphatidylcholine and unesterified fatty acids determined as described in the Experimental section. The percentage desaturation represents radioactivity in the linoleoyl moiety as a percentage of that in oleoyl and linoleoyl moieties.

Substrate supplied	Desaturation in 3- <i>sn</i> -phosphatidylcholine (%)	Position 1		Position 2	
		Proportion of total radioactivity (%)	Desaturation (%)	Proportion of total radioactivity (%)	Desaturation (%)
[^{14}C]Oleoyl-CoA	6.6	24.1 (26.4)	5.4 (3.5)	75.9 (73.6)	7.4 (5.6)
	15.3	24.6	13.0	75.4	15.9
	36.5	26.5	23.0	73.5	30.0
	64.9	25.2 (20.3)	44.2 (45.4)	74.8 (79.7)	54.5 (54.7)
	12.9	17.5	6.2	82.5	16.7
	35.5	15.5	20.3	84.5	34.8
[^{14}C]Dioleoyl phosphatidylcholine	10.9	55.1	8.8	44.9	12.3
	25.8	54.5 (57.2)	23.7 (22.1)	45.5 (42.8)	30.8 (26.1)

Table 6. *Inhibition of oleate desaturation by sn-glycero-3-phosphocholine*

Expt. a: [^{14}C]oleoyl-CoA (24 nmol) was incubated with microsomal preparations containing 0.12 μmol of 3-*sn*-phosphatidylcholine with and without *sn*-glycero-3-phosphocholine for 20 min. Expt. b: [^{14}C]dioleoyl phosphatidylcholine containing 26 nmol [^{14}C]oleate (1.29 $\mu\text{Ci}/\mu\text{mol}$) was added in ethanol to reaction mixtures with and without *sn*-glycero-3-phosphocholine containing microsomal preparations (0.2 μmol of 3-*sn*-phosphatidylcholine) and incubated for 50 min. Expt. c: microsomal preparations containing 0.19 μmol of 3-*sn*-phosphatidylcholine labeled *in situ* with [^{14}C]oleate by preincubation with [^{14}C]oleoyl-CoA as described in Table 4 were incubated in reaction mixtures containing different concentrations of *sn*-glycero-3-phosphocholine for 10 min. Details of the assay procedure and analyses are given in the Experimental section.

Expt.	Substrate supplied	Concentration of <i>sn</i> -glycero-3-phosphocholine (mM)	Proportion of radioactivity in linoleate 3- <i>sn</i> -phosphatidylcholine (%)	Radioactivity in chloroform phase (%)
a	[^{14}C]Oleoyl-CoA	0	35.8	98
		5	4.0	62
b	[^{14}C]Dioleoyl phosphatidylcholine	0	11.4	
		5	2.3	
c	[^{14}C]Oleoyl phosphatidylcholine	0	33.6	
		0.2	31.3	
		1.0	18.9	
		5.0	8.8	

and position 2, in parentheses, and the s.e.m. values for the eight analyses performed with fungal lipase in Table 5 were (in mol/100 mol): palmitate, 23.5 ± 0.7 (1.6 ± 0.1); stearate, 8.6 ± 0.6 (0.7 ± 0.1); oleate, 7.3 ± 0.2 (5.9 ± 0.2) and linoleate, 59.7 ± 1.3 (91.5 ± 0.3). These data reflect largely the composition of the 3-*sn*-phosphatidylcholine of mature safflower seed cotyledons added during the analyses as carrier.

Inhibition of oleate desaturation by sn-glycero-3-phosphocholine

Vijay & Stumpf (1971) reported that *sn*-glycero-3-phosphocholine inhibited oleate desaturation by microsomal preparations from safflower cotyledons and argued that, since this compound appeared to serve as an acceptor for oleate from oleoyl-CoA, the inhibition indicated that oleoyl-CoA was the substrate for oleate desaturation. In view of our present results the effect of *sn*-glycero-3-phosphocholine was re-examined. In contrast with the previous observation (Vijay & Stumpf, 1971), this compound inhibited to some extent the transfer of oleate from oleoyl-CoA to chloroform-soluble lipids (Table 6, Expt. a) However, more particularly this compound inhibited the appearance of label in the linoleoyl moiety of 3-*sn*-phosphatidylcholine when [^{14}C]oleoyl-CoA was supplied (Expt. a) and also when exogenous and endogenous [^{14}C]oleoyl phosphatidylcholine were substrates (Expts. b and c, respectively). No accumulation of water-soluble [^{14}C]linoleoyl esters occurred in reaction mixtures that contained *sn*-glycero-3-phosphocholine in Expt. a.

Discussion

It has been claimed (Vijay & Stumpf, 1971) or inferred (Ben Abdelkader *et al.*, 1973; Tremolières & Mazliak, 1974; Dubacq *et al.*, 1976; Kader, 1977) that oleoyl-CoA is the substrate for oleate desaturation in higher-plant microsomal fractions and that linoleate from linoleoyl-CoA is rapidly transferred to 3-*sn*-phosphatidylcholine. Our results with microsomal preparations of developing safflower seed cotyledons are inconsistent with this view. Rather the rapid incorporation of oleate from oleoyl-CoA into 3-*sn*-phosphatidylcholine, then the subsequent conversion of [^{14}C]oleate into [^{14}C]linoleate without any concomitant change in the specific radioactivity of the lipid, suggests that 3-*sn*-phosphatidylcholine is intimately involved in oleate desaturation. This same conclusion was reached previously from studies of oleoyl-CoA metabolism by microsomal preparations from the fungus *Neurospora crassa* (Baker & Lynen, 1971), the yeast *Torulopsis utilis* (Talamo *et al.*, 1973) and pea (*Pisum sativum*) leaf (Slack *et al.*, 1976). It is, however, contrary to that of Vijay & Stumpf (1971) who, like us, used microsomal preparations from cotyledons of developing safflower seed. To distinguish acyl thiol esters from acyl glycerol esters, Vijay & Stumpf (1971) used the method of Barron & Mooney (1968). Since, however, NaBH_4 under the conditions that they used reductively hydrolyses the acyl esters of polar lipids as well as acyl thiol esters (Nichols & Safford, 1973), it is conceivable that the amount of linoleate as a thiol ester could have been grossly overestimated at

the expense of linoleate esterified to 3-*sn*-phosphatidylcholine.

It is implicit that linoleoyl-CoA should be the product of oleoyl-CoA desaturase, but in studies claiming to show the presence of this enzyme in plant microsomal preparations, linoleoyl-CoA was not identified, since reaction mixtures were routinely saponified before analysis (see references above: Ben Abdelkader *et al.*, 1973; Tremolières & Mazliak, 1974; Dubacq *et al.*, 1976; Kader, 1977). Where products were analysed by chromatographic procedures the linoleate derived from oleoyl-CoA was found localized in 3-*sn*-phosphatidylcholine (Ben Abdelkader *et al.*, 1973; Dubacq *et al.*, 1976). We suggest, therefore, that it is questionable from the information presently available whether such an enzyme exists in plants.

In the present studies the conversion of [^{14}C]oleate in 3-*sn*-phosphatidylcholine into [^{14}C]linoleate was accompanied by a decline in the amount of oleate and a corresponding increase in that of linoleate in the lipid, and hence an estimate of total oleate desaturation based on the original specific radioactivity of the oleoyl-CoA supplied would be an underestimate of activity. That all oleoyl moieties of the lipid were not desaturated at the same rate was apparent from the decline in specific radioactivity of the [^{14}C]oleate of the lipid as the extent of desaturation became considerable. However, during the initial period of desaturation of endogenous [^{14}C]oleoyl phosphatidylcholine (Expt. a, Table 4) the specific radioactivity of the oleoyl moiety remained constant, and consequently an estimate of activity can be made. During the first 5 min period, 19.7% of the oleate in the lipid was desaturated, equal to a rate of 3.9%/min, assuming linearity. At zero time, oleate represented 18.6 mol/100 mol of the fatty acids in the lipid and hence 1 μmol of the lipid would have initially contained 372 nmol of oleate. The average rate of desaturation of 3.9%/min over the first 5 min, therefore, was equivalent to 14.5 nmol of oleate desaturated/min per μmol of 3-*sn*-phosphatidylcholine, or a rate that, if it had been maintained, would have desaturated all the oleate in the lipid in 25 min.

Our present finding that oleate at both positions 1 and 2 of 3-*sn*-phosphatidylcholine could be desaturated was in agreement with that of Pugh & Kates (1975) for the oleoyl phosphatidylcholine desaturase from *Candida lipolytica*, but not with that of Talamo *et al.* (1973), who concluded that the desaturation probably occurred exclusively at position 2 of the phospholipid in *Torulopsis utilis*. If, as our present studies suggest, oleoyl phosphatidylcholine is the precursor of linoleoyl phosphatidylcholine in developing safflower cotyledons, then it would seem reasonable to expect that desaturation would occur at both positions, since linoleate repre-

sents about 70% of the fatty acid of the lipid and occurs at both positions (see above). Furthermore, kinetic labelling studies *in vivo* indicate that, in linseed and soya-bean cotyledons, dioleoyl phosphatidylcholine is converted into dilinoleoyl phosphatidylcholine (Slack *et al.*, 1978), a route again consistent with oleate desaturation at both positions of the lipid.

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