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Evidence for the reversibility of the acyl-CoA:lysophosphatidylcholine acyltransferase in microsomal preparations from developing safflower (*Carthamus tinctorius* L.) cotyledons and rat liver

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Acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine occurs in the microsomal preparations of developing safflower cotyledons. Evidence is presented to show that the acyl exchange is catalysed by the combined back and forward reactions of an acyl-CoA:lysophosphatidylcholine acyltransferase (EC 2.3.1.23). The back reaction of the enzyme was demonstrated by the stimulation of the acyl exchange with free CoA and by the observation that the added CoA was acylated with acyl groups from position 2 of *sn*-phosphatidylcholine. Re-acylation of the, endogenously produced, lysophosphatidylcholine with added acyl-CoA occurred with the same specificity as that observed with added palmitoyl lysophosphatidylcholine. A similar acyl exchange, catalysed by an acyl-CoA:lysophosphatidylcholine acyltransferase, occurred in microsomal preparations of rat liver. The enzyme from safflower had a high specificity for oleate and linoleate, whereas arachidonate was the preferred acyl group in the rat liver microsomal preparations. The rate of the back reaction was 3–5% and 0.2–0.4% of the forward reaction in the microsomal preparations of safflower and rat liver respectively. Previous observations, that the acyl exchange in safflower microsomal preparations was stimulated by bovine serum albumin and *sn*-glycerol 3-phosphate, can now be explained by the lowered acyl-CoA concentrations in the incubation mixture with albumin and in the increase in free CoA in the presence of *sn*-glycerol 3-phosphate (by rapid acylation of *sn*-glycerol 3-phosphate with acyl groups from acyl-CoA to yield phosphatidic acid). Bovine serum albumin and *sn*-glycerol 3-phosphate, therefore, shift the equilibrium in acyl-CoA:lysophosphatidylcholine acyltransferase-catalysed reactions towards the rate-limiting step in the acyl exchange process, namely the removal of acyl groups from phosphatidylcholine. The possible role of the acyl exchange in the transfer of acyl groups between complex lipids is discussed.

Microsomal preparations from the developing cotyledons of oil-seed species (soya, safflower and sunflower) catalyse an exchange of acyl groups between acyl-CoA and position 2 of *sn*-phosphatidylcholine (Stymne & Glad, 1981; Stobart *et al.*, 1982; Stymne *et al.*, 1983; Stymne & Stobart, 1984). The acyl exchange does not require ATP and therefore appears not to involve a free fatty acid intermediate.

Linoleate, in developing oil-seeds, is synthesized by the direct desaturation of oleoyl phosphatidylcholine (Stymne & Appelqvist, 1978; Slack *et al.*, 1979; Stymne, 1980; Stymne *et al.*, 1983). The acyl exchange provides a mechanism whereby the oleate is transferred to position 2 of *sn*-phosphatidylcholine and the newly synthesized linoleate is returned to the acyl-CoA pool. We have shown that the fatty acid composition of the acyl-CoA pool is controlled by the acyl exchange and that this pool governs, to a large extent, the acyl components in the synthesized triacylglycerol (Stymne *et al.*, 1983; Stobart *et al.*, 1983).

The enzyme(s) involved in the acyl exchange process has yet to be elucidated. Roughan & Slack (1982) referred to the acyl exchange as catalysed by

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a novel enzyme, acyl-CoA:phosphatidylcholine acyltransferase, and we considered that an enzyme complex was probably involved (Stymne *et al.*, 1983). However, there have been reports that the transfer of acyl groups between phospholipids in rat liver microsomal preparations (Irvine & Dawson, 1979) and in homogenates of thymocytes (Trotter & Ferber, 1981) is catalysed by the reversal of an acyl-CoA:lysophosphatidylcholine acyltransferase (EC 2.3.1.23). The enzyme catalyses the acylation of lysophosphatidylcholine with acyl groups from acyl-CoA (see Van den Bosch *et al.*, 1972). The equilibrium of the reaction is normally far towards the synthesis of phosphatidylcholine, since it results in the cleavage of a high-energy thioester bond and the formation of an oxygen bond. High concentrations of CoA and phosphatidylcholine may, however, favour the reverse reaction. The acyltransferase is therefore a possible candidate for catalysing the acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine. The first stage in the exchange would be the removal of an acyl group (X) from position 2 of *sn*-phosphatidylcholine by the reversal of the acyltransferase reaction (eqn. 1):

$$\text{X-phosphatidylcholine} + \text{CoA} \rightarrow \text{lysophosphatidylcholine} + \text{X-CoA} \quad (1)$$

The re-acylation of the lysophosphatidylcholine from a pre-existing pool of acyl-CoA (Y) would occur by the forward reaction of the enzyme (eqn. 2):

$$\text{Y-CoA} + \text{lysophosphatidylcholine} \rightarrow \text{Y-phosphatidylcholine} + \text{CoA} \quad (2)$$

The back and forward reactions (eqns. 1 and 2), working in concert, would result in acyl exchange (eqn. 3):

$$\text{X-phosphatidylcholine} + \text{Y-CoA} \rightarrow \text{X-CoA} + \text{Y-phosphatidylcholine} \quad (3)$$

In incubation mixtures containing microsomal preparations from developing oil seeds, the added acyl-CoA would rapidly acylate the lysophosphatidylcholine that is produced in the back reaction (eqn. 1). The lysophosphatidylcholine would therefore be kept at a low concentration in the reaction mixture and so promote the equilibration of the fatty acids in the acyl-CoA with those in position 2 of *sn*-phosphatidylcholine.

We have now tested these proposals in microsomal preparations from developing safflower seed and rat liver. It is these results that are reported in the present paper.

Materials and methods

Chemicals

1-^{14}C-labelled fatty acids were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.) and [^{3}H]CoA was from New England Nuclear (Boston, MA, U.S.A.). Bovine serum albumin (fraction V, fatty-acid-free), CoA, phospholipase A_2 [from Indian-cobra (*Naja naja*) snake venom], palmitoyl lysophosphatidylcholine and various fatty acids were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). SEP-PAK C_{18} silica-gel cartridges were from Waters Associates (Farmington, MA, U.S.A.).

Heptadecanoyl-CoA, [1-^{14}C]stearoyl-CoA (octadecanoyl-CoA) (sp. radioactivity 1640d.p.m./nmol), [1-^{14}C]oleoyl-CoA (octadec-9-enoyl-CoA) (sp. radioactivity 2580d.p.m./nmol), [1-^{14}C]linoleoyl-CoA (octadeca-9,12-dienoyl-CoA) (sp. radioactivity 2390d.p.m./nmol), [1-^{14}C]linolenoyl-CoA (octadeca-9,12,15-enoyl-CoA) (sp. radioactivity 3410d.p.m./nmol), [1-^{14}C]eicosa-8,11,14-trienoyl-CoA (sp. radioactivity 1170d.p.m./nmol), and [1-^{14}C]arachidonoyl-CoA (eicosa-5,8,11,14-tetraenoyl-CoA) (sp. radioactivity 1340d.p.m./nmol) were synthesized from their mixed anhydrides as described by Sanchez *et al.* (1973). The [^{14}C]eicosatrienoyl-CoA and [^{14}C]arachidonoyl-CoA had a chemical and radiochemical purity of 95% as determined by t.l.c., g.l.c. and radio-g.l.c. The other acyl-CoAs had a purity greater than 98%. [^{3}H]CoA was mixed with unlabelled CoA to give a specific radioactivity of 3700d.p.m./nmol.

Microsomal preparations

Safflower (*Carthamus tinctorius* L.) plants, var. Gila (a high-linoleate variety), were grown from seed in a 16h photoperiod at 28°C and an 8h night at 22°C. Seeds were harvested 14–18 days after flowering, and the cotyledons were removed and stored on ice. All further procedures were carried out at 1–4°C. The cotyledons were ground in a mortar with 2 parts (w/v) of 0.1M-potassium phosphate buffer, pH7.2, containing 0.1% bovine serum albumin and 0.33M-sucrose. The homogenate was filtered through a double layer of Miracloth, diluted 5-fold with fresh grinding medium and centrifuged at 18000*g* for 20min. The supernatant was filtered through Miracloth and centrifuged at 105000*g* for 90min. The resulting microsomal pellet was resuspended in 0.1M-potassium phosphate buffer, pH7.2, and stored at −70°C until required.

Rat liver microsomal preparations were obtained from fed male Sprague–Dawley rats (weighing 135–140g) by homogenizing the liver in 10mM-potassium phosphate buffer, pH7.2, containing 0.25M-sucrose and 1mM-EDTA in a Potter–Elvehjem homogenizer. The homogenate was thereafter treated as described for the safflower microsomal preparation.

The safflower and rat liver microsomal preparations contained approx. 230 and 200nmol of phosphatidylcholine/mg of protein respectively.

Enzyme assays

Assays with microsomal preparations from safflower and rat liver were carried out at 30°C and 37°C respectively (unless otherwise stated) in a water bath with constant shaking. The incubation mixture contained bovine serum albumin, cofactors, substrates and microsomal preparation (at concentrations stated in the Tables and Figures) dissolved in 0.1M-potassium phosphate buffer, pH7.2, in a final volume of 1ml. Lysophosphatidylcholine was added as a sonicated solution (2nmol/μl) to the microsomal incubation mixture and preincubated at the desired temperature for 2min before the addition of acyl-CoA, The assay for acyl exchange was started by the addition of the microsomal preparation to the reaction mixture.

Analytical procedures

Reactions were terminated by the addition of 3ml of 0.15M-acetic acid, and the lipids were extracted in a modification (Stymne & Glad, 1981) of the medium described by Bligh & Dyer (1959). 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.) and 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 plate and either assayed for radioactivity by liquid-scintillation counting as described by Stymne & Appelqvist (1978) or transmethylated with sodium methoxide (Slack *et al.*, 1976). The fatty acid methyl esters were analysed by g.l.c. and quantified by using methylheptadecanoic acid as an internal standard or analysed by radio-g.l.c. (Stymne & Appelqvist, 1978) for the determination of the radioactivity in the individual fatty acids.

Acyl-CoA was purified by reversed-phase chromatography on SEP-PAK C_{18} silica-gel cartridges as previously described (Stymne & Glad, 1981; Stymne *et al.*, 1983). The fatty acids in the acyl-CoA were identified and quantified by g.l.c. analysis of their methyl esters by using heptadecanoyl-CoA as an internal standard (50nmol was added to the incubation mixture just before the extraction). In incubations with [^{14}C]oleoyl-CoA and [^{3}H]CoA the first eluate and the water rinse from the SEP-PAK column were regarded as the CoA fraction (see Stymne & Glad, 1981), and a portion (1/100th) was assayed for radioactivity. Likewise the radioactivity in a portion (1/25th) of the subsequent methanol/NH_3 eluate, which contained the acyl-CoA, was determined. The elution of acyl-CoA from the SEP-PAK column was often incomplete. Therefore the recovery of the [^{14}C]oleate in the acyl-CoA was determined by comparing the radioactivity with the actual mass of the oleate present. It should be noted that no dilution of the [^{14}C]oleate with non-radioactive oleate occurred in the acyl-CoA during incubation. The ^{3}H radioactivity in the acyl-CoA fraction was therefore adjusted accordingly.

Positional analysis of the fatty acids in phosphatidylcholine was carried out with phospholipase A_2 (Stymne *et al.*, 1983).

Lipid samples were assayed for radioactivity in PCS (Amersham/Searle)/xylene (2:1, v/v) in a Beckman LS-230 liquid-scintillation counter with an efficiency of 94% for ^{14}C and 30% for ^{3}H. All radioactivity counts were corrected for background and quenching. The radioactivity in ^{14}C and ^{2}H dual-isotope experiments was determined by the channels-ratio method.

Reproducibility of results

The results given for each experiment are for single measurements only. Each experiment, however, was repeated at least twice with different microsomal preparations and all gave similar results.

Results

The proposal that the acyl exchange was catalysed by the combined back and forward reactions of an acyl-CoA : lysophosphatidylcholine acyltransferase was tested with microsomal preparations from the developing seeds of safflower (*C. tinctorius* var. Gila). The microsomal membranes have an active acyl exchange, and linoleate is the major acyl group in position 2 of *sn*-phosphatidylcholine (palmitate, 2.2%; stearate, 0.7%; oleate, 2%; linoleate, 95.1%).

Effect of CoA on the acyl-exchange between acyl-CoA and phosphatidylcholine

A high concentration of free CoA should shift the equilibrium of the reaction, catalysed by the acyltransferase, towards the removal of acyl groups from phosphatidylcholine. Since the removal of acyl groups from phosphatidylcholine is the rate-limiting step in the exchange process, it should result in the more rapid production of lysophosphatidylcholine (eqn. 1), which in turn will be re-acylated in the relatively faster forward reaction (eqn. 2). The effect of CoA on the incorporation of [^{14}C]oleoyl-CoA into phosphatidylcholine was therefore investigated in the microsomal preparations. The results show that the incorporation of [^{14}C]oleate into phosphatidylcholine increased with increasing CoA (Table 1). The incorporation

Table 1. *Effect of CoA on the incorporation of [^{14}C]oleate from [^{14}C]oleoyl-CoA into various lipids in microsomal preparations of developing safflower cotyledons*

Microsomal preparations (0.2mg of microsomal protein) were incubated with [^{14}C]oleoyl-CoA (160nmol), bovine serum albumin (10mg) and CoA. The radioactivity incorporated into the various lipids was determined after 5min incubation at 30°C. For experimental details see the Materials and methods section. The results shown are from a representative experiment.

	[^{14}C]Oleate incorporated (nmol)		
Concn. of CoA (μM)	Phosphatidylcholine	Phosphatidylethanolamine	Unesterified fatty acids and other $CHCl_3$-soluble lipids
0	2.2	0.3	3.2
25	5.5	0.4	4.2
100	10.6	0.3	4.4
200	12.9	0.3	4.4
400	16.4	0.3	4.4
800	17.8	0.3	4.5

Table 2. *Utilization of [^{14}C]oleoyl-CoA and [^{3}H]CoA in microsomal preparations from developing safflower cotyledons*

Microsomal preparations (0.4mg of microsomal protein) were incubated with [^{14}C]oleoyl-CoA (200nmol) and bovine serum albumin (10mg) in the presence of [^{3}H]CoA (200nmol). At intervals the [^{14}C]oleate and [^{3}H]CoA were determined in acyl-CoA, CoA and phosphatidylcholine, as well as the mass and identity of the acyl groups in acyl-CoA. For experimental details see the Materials and methods section. The results shown are from a representative experiment.

	Incorporation of radioactivity (nmol)				Fatty acids in acyl-CoA (nmol)	
		CoA fraction				
Incubation time (min)	Acyl-CoA [^{3}H]CoA	[^{14}C]Oleate	[^{3}H]CoA	Phosphatidylcholine [^{14}C]Oleate	Oleate	Linoleate
0*	16	30	196	0.3	199	3
5	38	29	172	31	171	28
10	51	24	148	40	148	38
15	57	26	144	50	140	44
30	69	20	129	60	128	58

* About 5s.

of oleate in position 2 of *sn*-phosphatidylcholine was greater than 96% (results not shown). The addition of 25 μM- and 800 μM-CoA increased the incorporation 2.5- and 8-fold respectively (Table 1). At the higher CoA concentration all the fatty acids in position 2 of *sn*-phosphatidylcholine would have been exchanged within 13min of incubation if the incorporation rate observed in the first 5min had been maintained. The incorporation of [^{14}C]-oleate into phosphatidyletholamine was unaffected by the increase in the CoA.

Experiments were carried out to demonstrate that the incorporation of [^{14}C]oleate was by acyl exchange and that the 'added' CoA was acylated with acyl groups from position 2 of *sn*-phosphatidylcholine. Microsomal preparations were incubated with [^{14}C]oleoyl-CoA (200nmol) and [^{3}H]CoA (200nmol). At regular intervals the [^{14}C]oleate in phosphatidylcholine, the [^{3}H]CoA in CoASH and acyl-CoA and the mass and identity of fatty acids in acyl-CoA were determined (Table 2). It is evident from the results (see 'zero' sample, Table 2) that some cross-contamination occurred between the acyl-CoA and the free CoA. The [^{3}H]CoA in the acyl-CoA nonetheless increased considerably, particularly during the early periods of incubation, and after 30min was approx. 4-fold greater than in the zero sample. Associated with the increase in [^{3}H]CoA in the acyl-CoA was a decrease in the [^{3}H]CoA in the CoA. During the 30min incubation period 60nmol of [^{14}C]oleate was incorporated into phosphatidylcholine. The [^{14}C]oleate in phosphatidylcholine was closely matched by the mass of linoleate that appeared in the acyl-CoA and, to a slightly lesser extent, the [^{3}H]CoA in the acyl-CoA fraction. Similar results were obtained with other safflower preparations. The relationship between the acyl groups in acyl-

CoA and phosphatidylcholine and the incorporation of [^{3}H]CoA and [^{14}C]oleate into acyl-CoA and phosphatidylcholine respectively confirms acyl exchange and the participation of the back reaction of the acyltransferase.

Acyl specificity in the acylation of endogenous phosphatidylcholine and added lysophosphatidylcholine in safflower microsomal preparations

The specificity and selectivity for different species of acyl-CoA were measured with the endogenous microsomal phosphatidylcholine and added lysophosphatidylcholine. The specificity of the acyl transfer was similar for both acyl acceptors in the single and mixed acyl-CoA substrate experiments (Table 3). Oleoyl-CoA and linoleoyl-CoA were the preferred substrates in the acylation of both acyl acceptors. Linolenate was transferred at about half the rate of the oleoyl and linoleoyl substrates, and stearoyl-CoA was strongly selected against (Table 3). The rate of acylation of the added lysophosphatidylcholine with oleate and linoleate was extremely rapid with a specific activity of approx. 300nmol/min per mg of protein (Table 3), and this was some 30 times more active than the acyl exchange.

Effect of bovine serum albumin on the incorporation of [^{14}C]oleate into phosphatidylcholine in safflower microsomal preparations

Acyl exchange in microsomal preparations from developing soya-bean cotyledons was undetectable in the absence of bovine serum albumin (Stymne & Glad, 1981). The addition of 1% bovine serum albumin increased the acyl exchange 3-fold in developing safflower cotyledons (Stymne *et al.*, 1983). The effect of bovine serum albumin on acyl exchange and the acylation of added lysophosphatidylcholine was therefore determined in the safflower preparations (Table 4). The acyl exchange, with endogenous phosphatidylcholine, increased approx. 2- and 2.7-fold with 0.5% and 1% bovine serum albumin respectively, and declined slightly with 2% bovine serum albumin. The acylation of added lysophosphatidylcholine, on the other hand, rapidly decreased with increasing bovine serum albumin. The acylation of the exogenous acyl acceptor and the acyl exchange with phosphatidylcholine were similar with 2% bovine serum albumin.

Bovine serum albumin is known to bind to acyl-CoA (Hershenson & Ernst-Fonberg, 1983) and its presence in the incubation mixture would decrease the free acyl-CoA. If the acylating enzyme prefers free acyl-CoA, the treatment with bovine serum albumin would help to drive the acyltransferase reaction backwards. A possible explanation for the results presented in Table 4 is that, with a bovine serum albumin concentration of up to 1%, the back reaction of the acyltransferase is the rate-limiting step in the acyl exchange, whereas at 2% bovine serum albumin it is the forward reaction that is limiting. It should be noted, however, that the decreased rate of acylation of lysophosphatidylcholine with increased bovine serum albumin could, at least in part, be due to a limiting amount

Table 3. *Acyl-CoA specificity in the acylation of endogenous phosphatidylcholine via acyl exchange and in the acylation of added palmitoyl lysophosphatidylcholine in the microsomal preparations of developing safflower cotyledons*

In the acyl-exchange experiments, microsomal preparations (0.4mg of microsomal protein) were incubated for 10min with [^{14}C]acyl-CoA (160nmol) (or, when mixed substrates were used, 40nmol of each [^{14}C]acyl-CoA), bovine serum albumin (10mg) and CoA (200nmol). When lysophosphatidylcholine was used as the acyl acceptor, microsomal preparations (0.12mg of microsomal protein) were incubated with palmitoyl lysophosphatidylcholine (100nmol), [^{14}C]acyl-CoA (160nmol) (or 40nmol of each acyl-CoA in the case of the mixed substrates). The radioactivity in phosphatidylcholine was determined after 2min incubation. For experimental details see the Materials and methods section. The results shown are from a representative experiment.

[^{14}C]Acyl-CoA substrate	^{14}C-labelled fatty acids incorporated into phosphatidylcholine (nmol/min per mg of protein)	
Acyl acceptor ...	Endogenous phosphatidylcholine	Palmitoyl lysophosphatidylcholine
Single substrate		
Stearoyl-CoA	1	41
Oleoyl-CoA	11	294
Linoleoyl-CoA	8	301
Linolenoyl-CoA	5	205
Mixed substrates		
Stearoyl-CoA	0	5
Oleoyl-CoA	3	107
Linoleoyl-CoA	3	100
Linolenoyl-CoA	2	57

Table 4. *Effect of bovine serum albumin on the incorporation of [^{14}C]oleate into phosphatidylcholine with endogenous phosphatidylcholine and with added palmitoyl lysophosphatidylcholine in microsomal preparations of developing safflower cotyledons*

Incubations with endogenous phosphatidylcholine as the acyl acceptor were performed with 0.2mg of microsomal protein, CoA (200nmol) and [^{14}C]oleoyl-CoA (200nmol) for 5 min. Incubations with lysophosphatidylcholine contained 0.12mg of microsomal protein, palmitoyl lysophosphatidylcholine (150nmol) and [^{14}C]oleoyl-CoA (200nmol) for 2 min. Bovine serum albumin was added at the concentrations stated in the Table. For experimental details see the Materials and methods section. The results shown are from a representative experiment.

Concn. of bovine serum albumin (%, w/v)	[^{14}C]Oleate incorporated into phosphatidylcholine (nmol/min per mg of protein)	
	Endogenous phosphatidylcholine	Palmitoyl lysophosphatidylcholine
0	6	339
0.5	11	194
1.0	16	63
2.0	13	14

of acyl acceptor as a consequence of its binding to the albumin.

Acyl exchange in rat liver microsomal preparations

The above results strongly suggest that the acyl exchange in safflower microsomal preparations is catalysed by an acyl-CoA:lysophosphatidylcholine acyltransferase. It is therefore important to establish whether similar enzymes present in the membrane fractions of other organisms could also catalyse acyl exchange. The acyl-CoA:lysophosphatidylcholine acyltransferase has been extensively studied in rat liver microsomal preparations (Yamashita *et al.*, 1973, 1975; Miki *et al.*, 1977; Lands *et al.*, 1982) and is reported to be reversible (Irvine & Dawson, 1979). Acyl exchange was therefore investigated in preparations from rat liver.

Microsomal preparations were incubated with [^{14}C]arachidonoyl-CoA in the presence and in the absence of CoA. EDTA was added to the incubation mixture to inhibit the formation of lysophosphatidylcholine by the activity of phospholipase A_2 (Newkirk & Waite, 1973). The incorporation of [^{14}C]arachidonate into phosphatidylcholine was stimulated in the presence of CoA (1mM) (Fig. 1). After 40min and 135min incubation the stimulation was approx. 60% and 30% respectively. The stimulation by CoA was considerably less than that observed in the safflower microsomal preparations (Table 1). It should be noted, however, that substantial amounts of free CoA were found in the incubation mixtures, even in the absence of added CoA, which were due to the presence of an active acyl-CoA thioester hydrolase (see unesterified fatty acids, Fig. 1). Added CoA had little effect on the incorporation of [^{14}C]arachidonate into phosphatidylethanolamine (Fig. 1). Bovine serum albumin (1%) was added to all the reaction mixtures, since its omission resulted in a 10-fold increase in

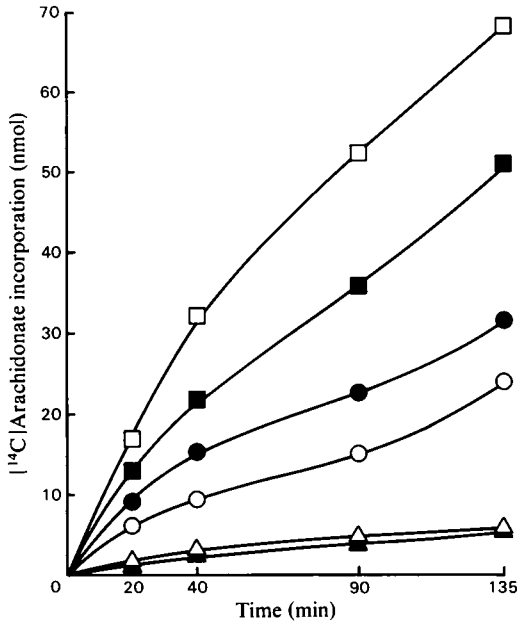

Fig. 1. *Effect of CoA on the incorporation of [^{14}C]arachidonate from [^{14}C]arachidonoyl-CoA into various lipids in rat liver microsomal preparations*

Microsomal preparations (1.5mg of microsomal protein) were incubated with [^{14}C]arachidonoyl-CoA (200nmol), EDTA (10μmol) and bovine serum albumin (10mg) in the presence (1μmol; ●, ▲ and ■) and in the absence (○, △ and □) of CoA at 30°C. The radioactivity in the various lipids was determined at regular intervals. For experimental details see the Materials and methods section. ● and ○, Phosphatidylcholine; ▲ and △, phosphatidylethanolamine; ■ and □, unesterified fatty acid. The results shown are from a representative experiment.

thioester hydrolase activity and caused the complete disappearance of the acyl-CoA substrate in a few minutes (results not shown).

To confirm that the acyl-CoA entered the phosphatidylcholine by acyl exchange, incubations were carried out with the microsomal preparations and [^{14}C]eicosatrienoyl-CoA in the presence of added CoA and bovine serum albumin. At regular intervals the radioactivity in phosphatidylcholine and the mass and identity of the fatty acids in the acyl-CoA were determined. Eicosatrienoate was chosen as the substrate since it is readily accepted by the acyltransferase (Lands *et al.*, 1982) and is almost absent from the microsomal phosphatidylcholine (about 1.5% of the acyl groups present). Thus any acyl groups in the acyl-CoA that are derived from phosphatidylcholine, via acyl exchange, can be accurately determined.

The results (Table 5) show that the eicosatrienoate was rapidly transferred to phosphatidylcholine, and to a lesser extent phosphatidylethanolamine, throughout the incubation period. More than 98% of the eicosatrienoate incorporated into *sn*-phosphatidylcholine was in position 2 (results not shown). Eicosatrienoate was also incorporated into other complex lipids, and this amounted to 11 nmol after 40 min incubation (results not shown). The eicosatrienoate in the acyl-CoA, on the other hand, substantially decreased, and associated with this was the rapid appearance of arachidonate. Small quantities of linoleate were also found in the acyl-CoA. Arachidonate and linoleate were the only fatty acids to increase in the acyl-CoA.

The total arachidonate and linoleate in the acyl-CoA up to 40 min incubation exceeded the [^{14}C]eicosatrienoate transferred to phosphatidylcholine and phosphatidylethanolamine. The discrepancy, however, was largely accounted for by the eicosatrienoate present in other complex lipids on the t.l.c. plates. The decline in fatty acid in acyl-CoA that occurred after 40 min incubation was due to the activity of a thioester hydrolase that acts on both the [^{14}C]eicosatrienoyl-CoA substrate as well as the exchanged acyl groups in acyl-CoA.

The results therefore show that the rat liver microsomal preparations catalyse the transfer of eicosatrienoate by acyl exchange to phosphatidylcholine and other lipids. The acyl exchange brings about the selective removal of arachidonate, and to a lesser extent linoleate, from the complex lipids, and these fatty acids re-acylate the CoA. It was also noted that if EDTA was omitted from the reaction mixture the transfer of [^{14}C]eicosatrienoate to phosphatidylcholine was slightly stimulated (approx. 15%) and that the arachidonate that appeared in acyl-CoA was only one-tenth of that found in the presence of EDTA (results not shown). These observations indicate that the higher phospholipase A_2 activity in the microsomal preparations in the absence of EDTA generates lysophosphatidylcholine and that this hinders the back reaction of the acyltransferase.

Acyl specificity in the acylation of endogenous phosphatidylcholine and added lysophosphatidylcholine in rat liver microsomal preparations

The specificity and rates of acyl transfer were measured with [^{14}C]eicosatrienoyl-CoA, [^{14}C]linoleoyl-CoA and [^{14}C]arachidonoyl-CoA with added lysophosphatidylcholine and also via the endogenous phosphatidylcholine. The microsomal preparations were incubated with acyl-CoA, which was presented either as a single or a mixed substrate (Table 6). The specificity for acyl-CoA species was similar for both the exogenous and endogenous acyl acceptor with the mixed acyl-CoA. The arachidonate was transferred 3–4 times faster than the eicosatrienoate and at a perhaps slightly greater rate than observed for linoleate. The transfer of eicosatrienoate to the endogenous phosphatidylcholine in the single-substrate experi-

Table 5. *Acyl exchange in microsomal preparations of rat liver*

Microsomal preparations (1.5 mg of microsomal protein) were incubated with [^{14}C]eicosatrienoyl-CoA (200 nmol), EDTA (10 μmol), bovine serum albumin (10 mg) and CoA (1 μmol) at 37°C. At regular intervals the [^{14}C]eicosatrienoate in the various lipids and the mass and identity of the fatty acids in the acyl-CoA were determined. For experimental details see the Materials and methods section. The results shown are from a representative experiment.

Incubation time (min)	[^{14}C]Eicosatrienoate incorporated (nmol)		Fatty acids in acyl-CoA (nmol)		
	Phosphatidylcholine	Phosphatidylethanolamine	Linoleate	Eicosatrienoate	Arachidonate
0*	0.5	0	0	212	0.5
20	18	2	3	167	24
40	27	4	5	159	35
80	37	5	6	79	25
120	46	8	6	36	17

* About 5s.

Table 6. *Acyl-CoA specificity in the acylation of endogenous phosphatidylcholine via acyl exchange and in the acylation of added palmitoyl lysophosphatidylcholine in microsomal preparations of rat liver*

In the acyl-exchange experiments microsomal preparations (1.5mg of microsomal protein) were incubated for 40min at 37°C with [^{14}C]acyl-CoA (200nmol) (or 60 nmol of each acyl-CoA when mixed substrates were used), bovine serum albumin (10mg), EDTA (10μmol) and CoA (1μmol). When added lysophosphatidylcholine was used as acyl acceptor, microsomal preparations (0.15mg of microsomal protein) were incubated with palmitoyl lysophosphatidylcholine (100nmol) and [^{14}C]acyl-CoA (180nmol) (or 60nmol of each acyl-CoA in the case of the mixed substrates). The radioactivity in phosphatidylcholine was determined after 2min incubation. For experimental details see the Materials and methods section. The results shown are from a representative experiment.

[^{14}C]Acyl-CoA substrate	^{14}C-labelled fatty acids incorporated in phosphatidylcholine (nmol/min per mg of protein)	
Acyl acceptor ...	Endogenous phosphatidylcholine	Palmitoyl lysophosphatidylcholine
Single substrate		
Linoleoyl-CoA	Not measured	66
Eicosatrienoyl-CoA	0.4	78
Arachidonoyl-CoA	0.3	125
Mixed substrates		
Linoleoyl-CoA	0.1	17
Eicosatrienoyl-CoA	0.1	24
Arachidonoyl-CoA	0.3	71

ments was slightly greater than for arachidonate. The specificity with exogenous lysophosphatidylcholine was, however, greater for arachidonate.

Discussion

The results confirm that the acyl exchange between acyl-CoA and position 2 of *sn*-phosphatidylcholine in safflower microsomal preparations is catalysed by the combined reverse and forward reactions of an acyl-CoA:lysophosphatidylcholine acyltransferase (EC 2.3.2.23). The results show that (1) acyl exchange is stimulated by the addition of free CoA and that the CoA is acylated with fatty acids derived from position 2 of *sn*-phosphatidylcholine and (2) re-acylation of phosphatidylcholine occurs with the same acyl specificity as the acylation of exogenous lysophosphatidylcholine.

The stimulation of acyl exchange with bovine serum albumin (Stymne & Glad, 1981; Stymne *et al.*, 1983) can now be explained. The bovine serum albumin lowers the concentration of free acyl-CoA in the incubation mixture and shifts the equilibrium of the acyltransferase towards the removal of fatty acids from phosphatidylcholine. The stimulation of acyl transfer from acyl-CoA to phosphatidylcholine that occurred in safflower microsomal preparations in the presence of *sn*-glycerol 3-phosphate (Stymne *et al.*, 1983) was only partly accounted for by transfer of diacylglycerol backbone, derived from phosphatidic acid, into phosphatidylcholine. A rapid increase in the free CoA in the incubation mixtures would occur, however, in the presence of *sn*-glycerol 3-phosphate, owing to the removal of acyl groups from acyl-CoA in the synthesis of phosphatidic acid (Stobart *et al.*, 1982, 1983). This would act similarly to bovine serum albumin and favour the back reaction of the acyltransferase. An acyl-CoA:lysophosphatidylcholine acyltransferase has been described in microsomal preparations from developing safflower seeds (Moreau & Stumpf, 1982). The activity of the enzyme was, however, only 0.1–1% of that reported in the present paper and its acyl specificity was quite different. We can offer no explanation for the discrepancy in these results. It is possible, however, that the practice of freezing the whole seed for storage before removal and homogenizing the cotyledons (Moreau & Stumpf, 1982) seriously affects enzyme activity. Certainly we have observed very poor incorporation of radioactive substrates *in vivo* in studies with previously frozen safflower seed (S. Stymne & A. K. Stobart, unpublished work).

We have previously shown that microsomal preparations of sunflower and safflower catalyse an equilibration between diacylglycerol and phosphatidylcholine (Stymne & Stobart, 1984; Stobart & Stymne, 1984). Evidence that this reaction may be catalysed by a CDP:1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) has been presented (Slack, 1983; Slack *et al.*, 1983). The enzyme provides phosphatidylcholine with oleate in both positions for subsequent desaturation and diacylglycerol rich in linoleate for the further synthesis of triacylglycerol. Oil-seeds, therefore, contain two enzymes, the acyl-CoA:lysophosphatidylcholine acyltransferase and the choline-

phosphotransferase, which, in reverse and forward reactions, are responsible for the entrance of oleate into phosphatidylcholine and the removal of the desaturated product for subsequent utilization in triacylglycerol production. The relative importance of the two enzymes in regulating the acyl quality of triacylglycerol has yet to be determined.

The experiments with the rat liver microsomal preparations show that acyl-CoA : lysophosphatidylcholine acyltransferase(s) from sources other than oil-seeds can operate in the back direction and catalyse acyl exchange, and this is in agreement with previous reports (Irvine & Dawson, 1979; Trotter & Ferber, 1981). The selectivity for arachidonate in the acylation of exogenous lysophosphatidylcholine and in the acyl exchange also confirms previous observations (Irvine & Dawson, 1979; Lands *et al.*, 1982). A possible role of the back reaction of the acyltransferase in animal cells in controlling the release of arachidonate from phospholipids for its subsequent involvement in prostaglandin synthesis has been suggested (Irvine, 1982). Elongation of endogenous acyl groups in phospholipid in the presence of bovine serum albumin without any equilibration with a fatty acid pool was demonstrated in rat brain microsomal preparations (Gan-Elepano *et al.*, 1981), and this could involve an acyl exchange catalysed by the acyltransferase. There are reports (Pugh & Kates, 1977, 1979) that liver microsomal preparations from starved–re-fed rats, in the presence of detergent, can desaturate eicosatrienoate in phosphatidylcholine to yield arachidonate. It is possible that acyl exchange could, if analogous to that in oil-seed microsomal preparations, provide phosphatidylcholine with eicosatrienoate for desaturation and the return of the arachidonate product to the acyl-CoA pool for utilization in the synthesis of other lipids. However, it should be noted that, when microsomal preparations from starved–re-fed rats were labelled *in situ* with [^{14}C]eicosatrienoyl phosphatidylcholine by incubation with [^{14}C]eicosatrienoyl-CoA in the presence of bovine serum albumin, the subsequent addition of NADH (which is necessary for desaturation) did not produce any [^{14}C]arachidonate in the phosphatidylcholine (S. Stymne, unpublished work). If the microsomal preparations were incubated with [^{14}C]eicosatrienoyl-CoA in the presence of NADH and unlabelled arachidonoyl-CoA, the [^{14}C]arachidonate that appeared in phosphatidylcholine was greatly decreased and there was a corresponding increase in [^{14}C]arachidonoyl-CoA (S. Stymne, unpublished work). These observations are in sharp contrast with those on the phosphatidylcholine desaturase in oil-seeds (Stymne & Appelqvist, 1978), and suggest that the eicosatrienoate is desaturated before it is incorporated into phospholipid and that the immediate product is arachidonoyl-CoA.

It is of interest to compare the ability of the safflower and liver preparations to catalyse the back reaction of the acyltransferase. The specific activities of the acylation of lysophosphatidylcholine and acyl exchange in the microsomal preparations from rat liver were 40 and 3% respectively of those for the safflower microsomal preparations. The differences in the specific activity of the acyltransferase from both sources probably reflect a dissimilar affinity of the enzyme for the substrates involved.

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References

Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
Gan-Elepano, M., Aeberhard, E. & Mead, J. F. (1981) *Lipids* **16**, 790–795
Hershenson, S. & Ernst-Fonberg, M. L. (1983) *Biochim. Biophys. Acta* **751**, 412–421
Irvine, R. F. (1982) *Biochem. J.* **204**, 3–16
Irvine, R. F. & Dawson, M. (1979) *Biochem. Biophys. Res. Commun.* **91**, 1399–1405
Lands, E. M., Inoue, M., Sugiura, Y. & Okuyama, H. (1982) *J. Biol. Chem.* **257**, 14968–14972
Miki, Y., Hasaka, K., Yamashita, S., Handa, H. & Numa, S. (1977) *Eur. J. Biochem.* **81**, 433–441
Moreau, R. A. & Stumpf, P. K. (1982) *Plant Physiol.* **69**, 1293–1297
Newkirk, J. D. & Waite, M. (1973) *Biochim. Biophys. Acta* **298**, 562–576
Pugh, E. L. & Kates, M. (1977) *J. Biol. Chem.* **252**, 68–73
Pugh, E. L. & Kates, M. (1979) *Lipids* **14**, 159–165
Roughan, P. G. & Slack, C. R. (1982) *Annu. Rev. Plant Physiol.* **33**, 97–132
Sanchez, M., Nicholls, D. G. & Brindley, D. N. (1973) *Biochem. J.* **132**, 697–706
Slack, C. R. (1983) *Proc. Annu. Symp. Bot. 6th* 40–55
Slack, C. R., Roughan, P. G. & Terpstra, J. (1976) *Biochem. J.* **155**, 71–80
Slack, C. R., Roughan, P. G. & Browse, J. (1979) *Biochem. J.* **179**, 649–656
Slack, C. R., Campbell, L. C., Browse, J. A. & Roughan, P. G. (1983) *Biochim. Biophys. Acta* **754**, 10–20
Stobart, A. K. & Stymne, S. (1984) *Planta* in the press
Stobart, A. K., Stymne, S. & Glad, G. (1982) *Proc. Int. Symp. Biochem. Metab. Plant Lipids* 257–261
Stobart, A. K., Stymne, S. & Glad, G. (1983) *Biochim. Biophys. Acta* **754**, 292–297
Stymne, S. (1980) Ph.D. Thesis, Swedish University of Agricultural Sciences

Stymne, S. & Appelqvist, L. A. (1978) *Eur. J. Biochem.* **90**, 223–229

Stymne, S. & Glad, G. (1981) *Lipids* **16**, 298–305

Stymne, S. & Stobart, A. K. (1984) *Biochem. J.* **220**, 481–488

Stymne, S., Stobart, A. K. & Glad, G. (1983) *Biochim. Biophys. Acta* **752**, 198–208

Trotter, J. & Ferber, E. (1981) *FEBS Lett.* **128**, 237–241

Van den Bosch, H., Van Golden, L. M. G. & van Deenen, L. L. M. (1972) *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **66**, 13–145

Yamashita, S., Hasada, K. & Numa, S. (1973) *Eur. J. Biochem.* **38**, 25–31

Yamashita, S., Nakaya, N., Miki, Y. & Numa, S. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 600–603