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SOME PROPERTIES OF DIACYLGLYCEROL ACYLTRANSFERASE IN A PARTICULATE FRACTION FROM MATURING SAFFLOWER SEEDS*

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Key Word Index—*Carthamus tinctorius*; Compositae; safflower; seeds; triacylglycerol synthesis; diacylglycerol acyltransferase.

Abstract—The activity of diacylglycerol acyltransferase of a subcellular particulate fraction from maturing safflower seeds was remarkably stimulated by the addition of 1, 2-diacylglycerols which were previously emulsified in a gelatin solution by sonication. Metal ions were inhibitory to the reaction. Deoxycholate and diisopropyl fluorophosphate were the most effective inhibitors. Sulfhydryl groups seemed to be of limited significance in the enzyme. Both 1, 2-dioleoyl-*sn*-glycerol and 2, 3-dioleoyl-*sn*-glycerol were good substrates of diacylglycerol acyltransferase, but the 1, 3-isomer did not serve as an acyl acceptor. The enzyme showed broad specificity for synthetic *rac*-1, 2-diacylglycerols containing various fatty acids. However, *rac*-1, 2-diacetylglycerol and *rac*-1, 2-dibutyrylglycerol, which are soluble in water, were ineffective. The enzyme exhibited no significant specificity for saturated and unsaturated fatty acyl-CoA thioesters as acyl donors. This suggests that the fatty acid composition at the 3-position of the glycerol molecule of safflower triacylglycerols may depend on the composition of the endogenous acyl-CoA pool.

INTRODUCTION

The biosynthesis of triacylglycerols (TG) occurs by the stepwise acylation of *sn*-glycerol-3-phosphate with long-chain fatty acyl-CoAs [1]. The last enzyme of this pathway, diacylglycerol acyl-transferase [acyl-CoA: 1,2-diacylglycerol *O*-acyltransferase (EC 2.3.1.20)], catalyses the following reaction: 1,2-diacylglycerol + acyl-CoA → TG + CoA. In animals, it is localized predominantly in microsomal subcellular fractions [2–6]. In maturing oil-rich seeds, TG's are formed in oleosomes (oil bodies) [7–10], while the presence of other organelles catalysing the biosynthesis of TG has been indicated by several workers [11–13]. It has previously been reported that a subcellular particulate fraction prepared from maturing seeds of safflower (*Carthamus tinctorius* L.) catalyses TG synthesis from oleoyl-CoA and that the addition of *rac*-1,2-diacylglycerols stimulates the reaction [13]. This preparation was not contaminated by oleosomes. One of the purposes of the work reported here has been to find substances or conditions which stimulate or control TG formation from diacylglycerols (DG) and acyl-CoAs *in vitro*, because the activity *in vitro* has been lower than that *in vivo* [13]. Another purpose of the present investigation has been to reveal the substrate specificity (for DG and acyl-CoAs) of diacylglycerol acyltransferase in the particulate fraction from maturing safflower seeds. In many vegetable fats and oils saturated fatty acids are preferentially esterified at the 1- and 3-positions of the glycerol molecule and unsaturated fatty acids at the 2-position [13, 14]. Likely origins for this non-random distribution are the esterification of *sn*-glycerol-3-phosphate to form phosphatidic acids and the esterification of DG to form TG's. Previous investigations of acyltransferases have dealt almost exclusively with those in mammalian tissues [6] and micro-organisms [15, 16].

In addition, a simple preparative procedure for 2, 3-dioleoyl-*sn*-glycerol is described.

RESULTS AND DISCUSSION

Effect of additives on lipid synthesis

In order to find factors regulating the biosynthesis of TG and other lipids *in vitro*, compounds shown in Table 1 were added to the incubation system and incorporation of [^{14}C]oleoyl-CoA was determined. Mg^{2+} and Ca^{2+} depressed TG synthesis, namely diacylglycerol acyltransferase activity, and stimulated the synthesis of compound lipids. Hg^{2+}, di-isopropyl fluorophosphate, bovine serum albumin and sodium deoxycholate were powerful inhibitors of triacylglycerol formation. Bovine serum albumin inhibited the incorporation of [^{14}C]oleoyl-CoA into all of the lipid classes. This seems to be due to the formation of a complex of oleoyl-CoA and bovine serum albumin. Data on the effects of the sulphydryl-binding reagents and dithiothreitol suggest that sulfydryl groups may not be important for diacylglycerol acyltransferase. Dithiothreitol significantly stimulated compound lipid synthesis. The radioactivity in compound lipids is

*Part 4 in the series "Lipid Metabolism in Safflower". For Part 3 see ref. [13]. In this paper, trivial names are used for fatty acids except for hexanoic, octanoic, decanoic and heptadecanoic acids.

Table 1. Effect of various additives on lipid synthesis

Additive	Concn (mM)	[^{14}C]Oleoyl-CoA incorporated (nmol/mg protein) Total lipids	TG	FA	DG	CL
None	—	2.82	1.27	0.64	0.63	0.27
Mg^{2+}	5	2.94	0.81	0.84	0.65	0.64
Ca^{2+}	5	2.43	0.55	0.75	0.54	0.60
Hg^{2+}	1	2.14	0.31	1.13	0.26	0.44
PCMB	1	2.28	0.82	0.78	0.42	0.26
NEMI	2	3.26	1.51	0.68	0.63	0.45
DIFP	5	0.84	0.14	0.25	0.19	0.26
EDTA	5	2.90	1.13	0.84	0.49	0.44
DTT	5	3.74	1.21	0.68	0.60	1.25
BSA	0.1%	0.50	0.04	0.26	0.09	0.12
DOC*	0.1%	1.86	0.07	1.40	0.21	0.17
Gelatin*	0.05%	5.08	3.01	0.74	1.02	0.31

rac-1, 2-Diacylglycerols prepared from olive oil were previously emulsified in water by sonication and the emulsion added to the incubation mixture described in the Experimental.

**rac*-1, 2-Diacylglycerols were previously emulsified in a 1% aqueous solution of sodium deoxycholate and a 0.5% aqueous solution of gelatin, respectively, by sonication. The final concentration of *rac*-1, 2-diacylglycerols was 0.32 mM.

TG, Triacylglycerols; FA, free fatty acids; DG, diacylglycerols; CL, compound lipids; PCMB, sodium *p*-chloromercuribenzoate; NEMI, *N*-ethylmaleimide; DIFP, di-isopropyl fluorophosphate; DTT, dithiothreitol; BSA, bovine serum albumin; DOC, sodium deoxycholate.

distributed among phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol. phosphatidic acid, lysophosphatidic acid and others [Ichihara, K. and Noda, M., unpublished]. Di-isopropyl fluorophosphate, one of the inhibitors for hydrolytic enzymes, was added to depress the activity of acyl-CoA hydrolase, but it inhibited diacylglycerol acyltransferase most strongly. It has been shown in ref. [13] that Triton X-100 inhibits diacylglycerol acyltransferase in safflower seeds. As shown in Table 1, the enzyme was strongly inhibited even with a mild detergent, sodium deoxycholate. When *rac*-1, 2-diacylglycerols, previously emulsified in a gelatin solution, were added to the assay system, a marked increase of TG formation was observed accompanying a considerable increase of DG formation. It was reported by Demant [17] that trioleoylglycerol droplets coated with gelatin by sonication were stable for a long time and were suitable as substrates for soluble and membrane-associated lipases. He observed that lipase activity was affected by the particle size of oil droplets and that only in the suspension with the smallest particle diameter was it possible to obtain an increase in reaction rate with lipase concentration over the entire range. It is considered that diacylglycerol acyltransferase of safflower seeds also requires the smallest possible particles of 1, 2-diacylglycerols for appearance of its high activity. On the basis of these data, DG's emulsified in a gelatin solution were added to the incubation mixtures in experiments described below.

Dependence of diacylglycerol acyltransferase activity on temperature

It has previously been reported that the biosynthesis of TG from [^{14}C]acetate *in vivo* is stimulated under a high temperature condition and the formation of DG and phospholipids is depressed under this condition [18]. As shown in Fig. 1, the activity of diacylglycerol acyltransferase significantly increases with increasing incubation temperature, while the biosynthesis of compound lipids was not so much affected by temperature. These data suggest that in intact seeds the acylation of *sn*-glycerol-3-phophate with acyl-CoAs occurs under both low and high temperature conditions and that they rapidly turn over, while the acylation of DG occurs at a relatively high temperature. This hypothesis agrees with the fact that maturation of safflower seeds, i.e. the biosynthesis and accumulation of TG's occur early in summer. In view of the metabolism of phospholipids in cell membranes, it is notable that phospholipid synthesis is almost independent of changes in environmental temperature. However, a possibility that glycerophosphate acyltransferase may be almost absent in the particulate fraction must also be taken into account.

Effect of 1, 2-*dioleoyl*-sn-*glycerol concentration on triacylglycerol formation*

Incorporation of [^{14}C]oleoyl-CoA into TG's as a function of the concentration of added 1, 2-dioleoyl-

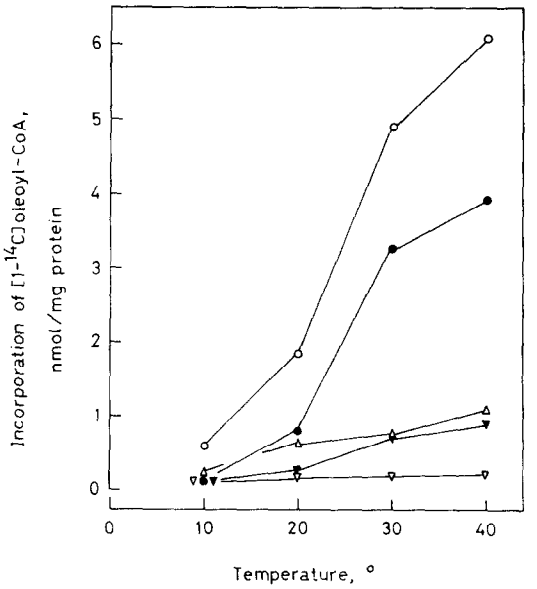

Fig. 1. Effect of temperature on incorporation of [1-^{14}C]oleoyl-CoA into lipids. (○) Total lipids, (●) triacylglycerols, (△) free fatty acids, (▼) diacylglycerols, (▽) compound lipids.

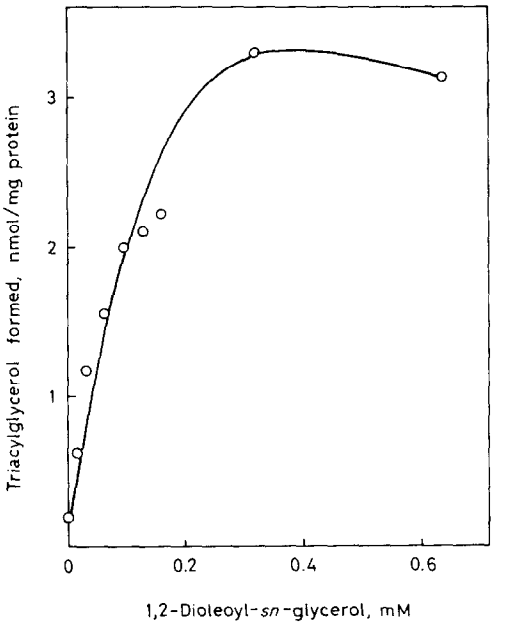

Fig. 2. Dependence of diacylglycerol acyltransferase on 1, 2-dioleoylglycerol concentration.

sn-glycerol is shown in Fig. 2. It can be seen that when dioleoylglycerol is omitted from the incubation mixture, there is a minimal but definite incorporation of [^{14}C]oleoyl-CoA, indicating the utilization of endogenous 1, 2-diacylglycerols present in the subcellular particulate preparation. When the concentration of 1, 2-dioleoyl-*sn*-glycerol was increased to 0.32 mM, the reaction rate reached a maximum. A low 1, 2-diacylglycerol level in subcellular particles is estimated from Fig. 2. Only a slight amount of 1, 2-diacylglycerols was actually detected both in the extract of the subcellular fraction and in lipids extracted from maturing seeds [Ichihara, K. and Noda, M., unpublished]. TG's *in vivo* were rapidly accumulated in maturing seeds 12–21 days after flowering [18], when the highest activity of fatty acid synthesis was also observed [19, 20]. It is described in ref. [18] that in safflower seeds 15 days after flowering [^{14}C]acetate has been incorporated into DG's most rapidly of all the lipid classes. These results suggest a rapid turnover of 1, 2-diacyl-*sn*-glycerols which are the precursors not only of TG but also of phospholipids.

Substrate specificity of diacylglycerol acyltransferase for dioleoylglycerol isomers

Table 2 clearly shows that diacylglycerol acyltransferase is not able to distinguish between 1,2-dioleoyl-*sn*-glycerol and 2,3-dioleoyl-*sn*-glycerol. Three dioleoylglycerol preparations, 1, 2-dioleoyl-*sn*-glycerol, 2,3-dioleoyl-*sn*-glycerol and *rac*-1,2-dioleoylglycerol, were equally effective as acyl acceptors. As the presence of 2, 3-diacyl-*sn*-glycerols *in vivo* has not yet been confirmed definitely, the participation of the 2, 3-isomers *in vivo* is questionable. However, it has become feasible to use the racemic compounds, which are easily prepared from TG's, as acyl acceptors. 1, 3-Dioleoylglycerol was ineffective. Exogeneous 2-oleoylglycerol is acylated with [^{14}C]oleoyl-CoA by the subcellular fraction to form 1, 2-dioleoyl-*X*-glycerol [Ichihara, K. and Noda, M., unpublished]. These data indicate that diacylglycerol acyltransferase of safflower seeds transfers acyl moieties of acyl-CoAs to the primary alcohol of acylglycerols on the lipid–water surface of emulsion. However, the presence of monoacylglycerol acyltransferase also must be considered.

Substrate specificity of diacylglycerol acyltransferase for rac-1, 2-*diacylglycerols with different fatty acyl chains*

Diacylglycerol acyltransferase activity was determined in the presence of various synthetic *rac*-1, 2-diacylglycerols and [^{14}C]oleoyl-CoA at two different concentrations of DG. As shown in Table 3, broad acyl-acceptor specificity was observed. 1, 2-Dihexanoylglycerol was the most effective acceptor of 1, 2-diacylglycerols tested. 1, 2-Dihexanoylglycerol and 1, 2-dioctanoylglycerol were more effective in low concentration than in high concentration. It is noteworthy that water-soluble 1, 2-diacylglycerols, 1, 2-diacetylglycerol and 1, 2-dibutyrylglycerol, are completely inactive as acyl acceptors. 1, 2-Dioctanoylglycerol, 1, 2-didecanoylglycerol, 1, 2-dilauroylglycerol and 1, 2-dimyristoylglycerol were good acyl acceptors. 1, 2-Distearoylglycerol and 1, 2-diarachidoylglycerol were ineffective. Although it was possible to disperse these DG's in a gelatin solution at 70–80°, the emulsions obtained became relatively unstable when they were cooled to room temperature. However, it seems that the ineffectiveness of these DG's is not necessarily due to insufficient emulsification, and it will reflect the specificity of diacylglycerol acyltransferase for these DG's to some

Table 2. Substrate specificity of diacylglycerol acyltransferase for dioleoylglycerol isomers

Dioleoylglycerol	Concn (mM)	[^{14}C]Oleoyl-CoA incorporated into triacylglycerols (nmol/mg protein)
Experiment 1		
Control	0.0	0.22
sn-1, 2-DG*	0.064	0.94
	0.32	2.01
rac-1, 2-DG	0.064	1.04
	0.32	1.92
1, 3-DG	0.064	0.34
	0.32	0.30
Experiment 2		
Control	0.0	0.71
sn-2, 3-DG	0.064	1.33
	0.32	1.79
rac-1, 2-DG	0.064	1.42
	0.32	2.11

*DG, Dioleoylglycerol.

Table 3. Substrate specificity of diacylglycerol acyltransferase for *rac*-1, 2-diacylglycerols with different fatty acid chains

rac-1, 2-Diacylglycerol	[^{14}C]Oleoyl-CoA incorporated into triacylglycerols (nmol/mg protein)	
	Concn of diacylglycerols	
	0.064 mM	0.32 mM
Control	0.71	
Diacetyl	0.70	0.73
Dibutyryl	0.77	0.87
Dihexanoyl	2.96	2.45
Dioctanoyl	1.77	1.27
Didecanoyl	2.01	2.04
Dilauroyl	1.40	2.25
Dimyristoyl	0.94	1.70
Dipalmitoyl	1.06	1.22
Distearoyl	0.67	0.80
Diarachidoyl	0.66	0.56
Dioleoyl	1.22	2.04
Dilinoleoyl	1.25	1.92

extent. 1, 2-Diacylglycerols containing unsaturated long-chain fatty acids, oleic and linoleic acids, were as good acyl acceptors as 1, 2-diacylglycerols containing saturated medium-chain fatty acids. There was no difference in incorporation between the two unsaturated 1, 2-diacylglycerols. That is to say, degree of unsaturation of fatty acid moieties in 1, 2-diacylglycerols is not significant for recognition of acyl acceptors by this acyltransferase. The effectiveness of *rac*-1, 2-diacylglycerols prepared from olive oil was nearly equal to that of the synthetic unsaturated *rac*-1, 2-diacylglycerols. The effectiveness of 1, 2-diacylglycerols containing medium-chain or unsaturated long-chain fatty acids seems to be explicable by the hydrophilic properties and mp's of the compounds.

It has been indicated that the biosynthesis of TG's in maturing oil-rich seeds [8, 21–23] proceeds according to the glycerol phosphate pathway [1] which has been elucidated in mammalian tissues, while Slack *et al.* [24, 25] and Appelqvist *et al.* [10, 26] have proposed an alternative route by which 1, 2-diacylglycerols are formed from phosphatidylcholines. The participation of phosphatidylcholines in the biosyn-

thesis of TG's is an important problem that should be investigated in further detail.

Acyl-donor specificity of diacylglycerol acyltransferase

The acyl-CoA dependence of diacylglycerol acyltransferases from rat fat cells [4], lactating rat mammary gland [27], lactating cow mammary gland [28] and rat liver [29] could not be related directly to the critical micellar concentration of acyl-CoA thioesters but the acyltransferases did indicate rather broad specificity. The specificity of diacylglycerol acyltransferase of safflower seeds for the fatty acid composition of acyl-CoA is shown in Fig. 3. Safflower diacylglycerol acyltransferase exhibited no significant specificity for saturated and unsaturated fatty acyl-CoAs, although linoleoyl-CoA was less effective at the low concentration. It may be preferable to use a mixture of acyl-CoAs as an acyl donor in order to compare the practical effectiveness of the thioesters with each other.

According to ref. [18], the fatty acid composition of the 3-position of safflower TG's seems to be more randomized than the 1- and 2-positions, and linoleic acid comprises over 70% of fatty acids synthesized *de novo* from [^{14}C]acetate in maturing seeds 15–18 days after flowering. It appears reasonable to consider that the fatty acid composition of the 3-position of safflower TG's may depend on the composition of the endogenous acyl-CoA pool. In view of the fact that TG's serve as an energy-storage material, the broad substrate specificity of diacylglycerol acyltransferase for both acyl acceptors and acyl donors is reasonable. In contrast to the 3-position of TG's saturated fatty acids were not detectable in the 2-position and only linoleic acid (91.9%) and oleic acid (8.1%) were esterified [18]. The fatty acid composition of the 2-position will be strictly controlled by 1-acylglycerophosphate acyltransferase.

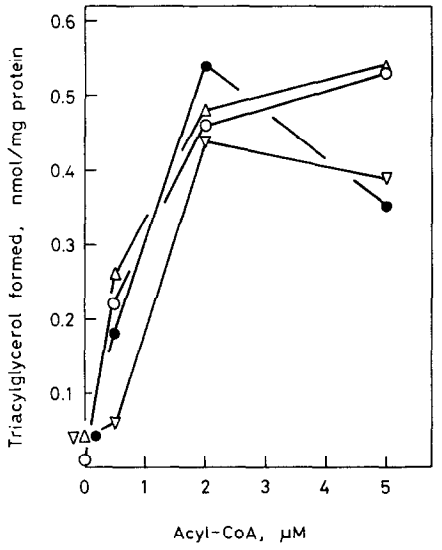

Fig. 3. Acyl-donor specificity of diacylglycerol acyltransferase. (○) Palmitoyl-CoA, (△) stearoyl-CoA, (●) oleoyl-CoA, (▽) linoleoyl-CoA.

It was reported that in Avocado microsomes little of the acyl moieties of acyl-ACPs was transferred to the polar lipids but extensive transfer to TG occurred [12]. The significance of acyl-ACPs for TG synthesis *in vivo* is not clear yet.

Preparation of 2, 3-*dioleoyl*-sn-*glycerol*

2, 3-Diacyl-*sn*-glycerols can be prepared from L-arabinose via L-mannitol [30–32]. In the present study, 2, 3-dioleoyl-*sn*-glycerol has been prepared from trioleoylglycerol by means of enzymatic and chemical techniques, which were satisfactory methods to obtain a relatively small amount of the 2, 3-isomer. The scheme is illustrated in Fig. 4.

EXPERIMENTAL

Preparation of simple TG's. Glycerol dried over P_2O_5 and satd fatty acids (C_8–C_{20}) (6 mol/mol glycerol) were heated at 180° for 2 hr and at 235° for 1 hr under red. pres. In the case of octanoic and decanoic acids, the reaction was carried out at a lower temp. The reaction mixtures were extracted with Et_2O or $CHCl_3$ (for TG's containing long-chain fatty acids) and washed with 5% Na_2CO_3 to remove free fatty acids. The organic layer was washed with H_2O and concd to dryness. The residue was crystallized from Me_2CO (for TG's containing medium-chain fatty acids), $CHCl_3$–Me_2CO (1:1) (for TG's containing long-chain fatty acids) or EtOH (−30°, for trioctanoylglycerol). Trioleoylglycerol and trihexanoylglycerol were synthesized from glycerol and the corresponding acid chlorides. Trilinoleoylglycerol was isolated from safflower oil by TLC on $AgNO_3$–Si gel G (1:9) (C_6H_6–Et_2O, 19:1).

Preparation of triacylglycerols as standard substances for GC and TLC. *rac*-1, 2-Dihexanoyl-3-palmitoylglycerol, *rac*-1,2-dihexanoyl-3-heptadecanoylglycerol, *rac*-1,2-dihexanoyl-3-stearoylglycerol, *rac*-1,2-dihexanoyl-3-oleoylglycerol and *rac*-1, 2-dihexanoyl-3-linoleoylglycerol were prepared from

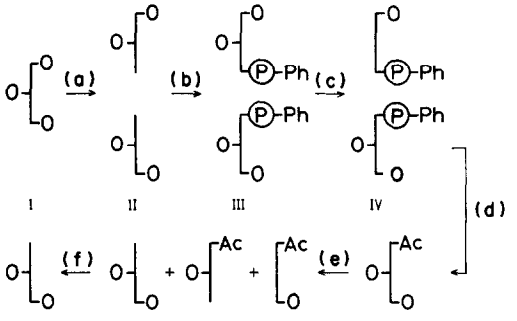

Fig. 4. Preparation of 2, 3-dioleoyl-*sn*-glycerol from trioleoylglycerol. I, Trioleoylglycerol; II, 1, 2-dioleoyl-*sn*-glycerol and 2, 3-dioleoyl-*sn*-glycerol; III, 1, 2-dioleoyl-*sn*-glycero-3-phosphophenol and 2, 3-dioleoyl-*sn*-glycero-1-phosphophenol; IV, 1-oleoyl-*sn*-glycero-3-phosphophenol and 2, 3-dioleoyl-*sn*-glycero-1-phosphophenol (the latter phospholipid was separated from the lysophospholipid by TLC); V, 1-acetyl-2, 3-dioleoyl-*sn*-glycerol; VI, 1-acetyl-3-oleoyl-*sn*-glycerol, 1-acetyl-2-oleoyl-*sn*-glycerol and 2, 3-dioleoyl-*sn*-glycerol; VII, 2, 3-dioleoyl-*sn*-glycerol. (a) Pancreatic lipase, (b) phenyl dichlorophosphate, (c) phospholipase A, (d) acetic acid–acetic anhydride, (e) ethyl magnesium bromide, (f) prep. TLC. O, Oleoyl; Ac, acetyl; Ⓟ–Ph, phosphophenol.

hexanoylchloride and corresponding *rac*-1-acylglycerols which were prepared by acylation of glycerol.

Preparation of DG's. TG's were partially hydrolysed by pancreatic lipase and the *rac*-1, 2-diacylglycerols were purified by the procedure of ref. [13]. *rac*-1, 2-Diacylglycerols from olive oil was also prepared by the same procedure. As tripalmitoylglycerol, tristearoylglycerol and triarachidoylglycerol were difficult to emulsify at 37°, they were hydrolysed with a thermostable lipase at high temp. They were sonicated in 0.2 M Tris–HCl buffer (pH 7.5) containing 0.5% gelatin at 60–80°. Thermostable lipase from *Pseudomonas fluorescence* was added to the TG emulsions and sonication repeated. The reaction mixtures were incubated at 60–75° for 5 min and extracted with $CHCl_3$. *rac*-1, 2-Diacylglycerols were purified by TLC on Si gel G (C_6H_6–Et_2O, 17:3).

1, 2-Dioleoyl-*sn*-glycerol was prepared by the hydrolysis of 1, 2-dioleoyl-*sn*-glycero-3-phosphocholine, which was prepared by the procedures of refs. [33–36], with phospholipase C from *Clostridium welchii* [37].

2, 3-Dioleoyl-*sn*-glycerol was prepared from *rac*-1, 2-dioleoylglycerol obtained from the partial hydrolysate of trioleoylglycerol (see Fig. 4). The racemic glyceride was converted to *rac*-1-phosphatidylphenol by the method of ref. [38]. Commercially available phospholipase A of *Crotalus adamanteus* (68 mg/g phosphatidylphenol) was dissolved in 0.2 M Tris–HCl buffer (pH 7.5) and heated at 95° for 2 min. A ppt was removed by centrifugation and the supernatant was added to an Et_2O soln of phosphatidylphenol. After full digestion for 5 days, 2, 3-dioleoyl-*sn*-glycero-1-phosphophenol, being unreactive to phospholipase A, was separated from lysophosphatidylphenol by CC on Si gel (phosphatidylphenol was eluted with $CHCl_3$–MeOH, 9:1) and TLC on Si gel G ($CHCl_3$–MeOH–HOAc, 85:15:1). Purified *sn*-1-phosphatidylphenol was converted to 1-acetyl-2, 3-dioleoyl-*sn*-glycerol by the method of ref. [39]. When this TG preparation was treated with EtMgBr [40], three DG's, 1-acetyl-2-oleoyl-*sn*-glycerol, 1-acetyl-3-oleoyl-*sn*-glycerol and 2, 3-dioleoyl-*sn*-glycerol, were obtained. 2, 3-Dioleoyl-*sn*-glycerol was separated from the other DG's by TLC on Si gel G (hexane–Et_2O, 3:2).

1, 3-Dioleoylglycerol was prepared by isomerization of *rac*-1, 2-dioleoylglycerol. A hexane soln of *rac*-1, 2-dioleoylglycerol was applied to a Si gel column and allowed to stand for 2 days. *rac*-1, 2-Dioleoylglycerol and 1, 3-dioleoylglycerol were eluted as a mixture with hexane–Et_2O (9:1). The latter DG was isolated from the mixture by TLC on Si gel G (C_6H_6–Et_2O, 17:3).

Preparation of acyl-CoAs and purification of gelatin. Unlabelled acyl-CoA thioesters were prepared by the procedure of ref. [41], and their concns were determined spectrophotometrically [42]. [1-^{14}C]Oleoyl-CoA was purchased from New England Nuclear (Boston, U.S.A.). Gelatin was purified by dialysis [17].

Enzyme preparation. Maturing safflower seeds 16–18 days after flowering were homogenized in 0.1 M Tris–HCl buffer (pH 7.6) containing 0.4 M sorbitol and 1 mM EDTA with a Potter–Elvejhem homogenizer. The homogenate was filtered through two layers of cotton cloth. The filtrate was centrifuged at 3000 *g* for 7 min, and the pellet obtained suspended with a small Potter–Elvejhem homogenizer in the same buffer used for homogenization.

Assay conditions. DG's were previously emulsified in H_2O with or without 0.5% gelatin by sonication. A typical assay system contained 50 mM Tris–HCl buffer (pH 7.6), 0.4 M sorbitol, 1 mM EDTA, 1.6 μM [1-^{14}C]oleoyl-CoA (50 Ci/mol), 0.1 ml DG emulsion and an aliquot of the pellet suspension. The final vol. was 1 ml and incubation was carried out at 30° for 20 min, unless otherwise stated. Lipids were extracted by the method of ref. [43] and separated into TG's, free fatty acids, DG's and compound lipids by TLC on Si gel G (hexane–Et_2O–HOAc, 70:30:1). The results shown in Fig. 2 and Tables 2 and 3 were obtained using labelled lipids which were separated into TG's and a mixture of other lipid classes by TLC on Si gel G (hexane–C_6H_6, 3:7). The radioactivity was measured by the procedure described in ref. [18].

Assay for acyl-donor specificity. The reaction mixture contained 50 mM Tris–HCl buffer (pH 7.6), 0.4 M sorbitol, 1 mM EDTA, 50 μM *rac*-1, 2-dihexanoylglycerol which was previously emulsified in a 0.5% aq. soln of gelatin, acyl-CoA and an aliquot of the pellet suspension in a final vol. of 10 ml. The final concn of gelatin was 0.05%. After incubation at 30° for 7 min, *rac*-1, 2-dihexanoyl-3-heptadecanoylglycerol was added to the incubation mixture as int. standard. Lipids were extracted by the method of ref. [43], and *rac*-1, 2-dihexanoyl-3-acylglycerols formed were separated from endogenous TG's with three long-chain fatty acyl groups by TLC on Si gel G (hexane–Et_2O, 4:1). The isolated *rac*-1, 2-dihexanoyl-3-acylglycerols were directly determined by GC under the following conditions: column packing, 5% SE-30; carrier gas, He (60 ml/min); column temp., isothermal 285°. Control incubations were conducted in the absence of acyl donors.

Protein. Determined by the method of ref. [44] with bovine serum albumin as standard.

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