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FORMATION OF PHOSPHOLIPIDS FROM *sn*-GLYCEROL-3-PHOSPHATE AND FREE FATTY ACIDS OR THEIR DERIVATIVES BY HOMOGENATES OF SOYBEAN COTYLEDONS

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Key Word Index—*Glycine max*; Leguminosae; soybean; acyltransferase activity; formation of phosphatidates and acylglycerols; cotyledon development; seed germination; fatty acid specificity; intracellular distribution.

Abstract—Cell-free homogenates of soybean cotyledons contain a *sn*-glycerol-3-phosphate acyltransferase system which incorporated [U-^{14}C]-*sn*-glycerol-3-phosphate into 5 labelled lipids when incubated with palmitic acid in the presence of ATP and CoA. In decreasing order of incorporation of label, the lipids were: *lyso*phosphatidic acid, monoacylglycerol, phosphatidic acid, diacylglycerol and triacylglycerol. The substrate specificity of the acyltransferase system was investigated with the fatty acids shown in order of decreasing rates of reaction; palmitate > stearate > oleate > linoleate > linolenate > laurate. Making these acids more soluble as triethanolamine salts or as polyoxyethylene sorbitan esters did not greatly enhance these rates of reaction. Activity was found in a 10 000 *g* pellet containing plastids, mitochondria and glyoxysomes and also in the lipid layer; the activity in these particulate fractions was enhanced by the addition of cytosol which itself had little activity when gentle methods of cell disruption were used. During cotyledon development the total acyltransferase activity increased, although its specific activity slowly declined due to more rapid synthesis of other proteins. During germination total activity decreased but there was a transient increase in specific activity due to more rapid degradation of other proteins.

INTRODUCTION

Kennedy [1] has shown that the biosynthesis of triacylglycerols in animal tissues involves the conversion of one molecule of *sn*-glycerol-3-phosphate and two fatty acyl-CoA molecules to *sn*-1,2-diacylglycerol-3-phosphate, also known as phosphatidic acid, by an enzyme system known as *sn*-glycerol-3-phosphate acyltransferase*. In animal tissues [2, 3] and in yeast [4], it is thought that there may be two acyltransferases involved, the first catalysing the formation of the mono-acyl derivative, *lyso*phosphatidic acid, which is then converted by a second acyltransferase to the diacyl derivative. In Kennedy's biosynthetic sequence [1], a phosphatase then converts phosphatidic acid to *sn*-1,2-diacylglycerol which undergoes a further esterification in the presence of a third molecule of fatty acyl-CoA and yet another acyltransferase to form triacylglycerol. The studies of Dybing and Craig [5] with flax embryos, of Gurr *et al.* [6] with developing seeds of *Crambe abysinnica*, of Harwood *et al.* [7] with maturing castor-bean seeds, and of Barron and Stumpf [8] with avocado mesocarp, suggest that the pathway of triacylglycerol formation in plant tissues involves the intermediate formation of phosphatidic acid and is, possibly, the same as postulated by Kennedy [1]. Evidence that this pathway also operates in soybean cotyledons was obtained by Wilson and Rinne [9] who showed, using intact cotyledons, that a number of phospholipids, including phosphatidic acid, could be metabolized to diacylglycerol which could then be reconverted to phospholipid or acylated to form triacylglycerol.

The results reported in this paper demonstrate the presence of a *sn*-glycerol-3-phosphate acyltransferase system in crude, cell-free homogenates of soybean cotyledons and support the view that the pathway of triacylglycerol formation in soybean cotyledons is as postulated by Kennedy for animal tissues. In one experiment, TLC was used to identify labelled products of the reaction. It was found that [U-^{14}C]-*sn*-glycerol-3-phosphate, in the presence of palmitate, CoA and ATP, was incorporated into both *lyso*phosphatidic and phosphatidic acids as well as mono-, di- and tri-acylglycerols. The isolation of these labelled products indicates that the homogenates contain all the enzymes mentioned above and, in addition, must also contain an ATP-dependent fatty acyl-CoA synthetase and a phosphatase which can dephosphorylate *lyso*phosphatidic acid. In routine assays, however, no attempt was made to isolate individual products or determine the proportion of labelled substrate incorporated into each; thus, the results of routine assays indicate only the amount of *sn*-glycerol-3-phosphate which has undergone the initial acylation to form *lyso*phosphatidic acid.

A study of acyltransferase activity during seed germination indicated that this enzymic system may play a role in the regulation of lipid biosynthesis.

* *Abbreviations:* Acyltransferase or *sn*-glycerol-3-phosphate acyltransferase, acyl-CoA: *sn*-glycerol-3-phosphate-*O*-acyltransferase (EC 2.3.1.15); fatty acyl-CoA synthetase, acid: CoA ligase (AMP) (EC 6.2.1.3).

RESULTS AND DISCUSSION

Cofactor requirements and optimum assay conditions for sn-*glycerol-3-phosphate acyltransferase*

Filtered homogenate was assayed as described in Experimental and the enzymic acylation of *sn*-glycerol-3-phosphate progressed linearly with time for at least 1 hr. Enzymic acylation sometimes progressed linearly with enzyme concentration (Fig. 1). However, more often, doubling the amount of protein in the incubation mixture would more than double the amount of *sn*-glycerol-3-phosphate acylated over the same period of time (Fig. 1). We could find no explanation for this deviation from linearity; experiments in which bovine serum albumin, acyl-carrier protein (ACP), thiols, CTP or additional amounts of cofactors (CoA and ATP) were added did not restore linearity. Dialysis led to an appreciable loss of activity which was only partly restored on the addition of thiols, CoA and ATP.

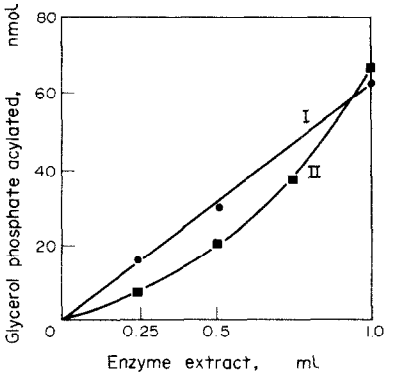

Fig. 1. Effect of enzyme concentration on *sn*-glycerol-3-phosphate acyltransferase activity. Reaction mixtures were incubated for 30 min and the ^{14}C-labelled products determined as described in Experimental using *sn*-glycerol-3-phosphate (0.1 μCi; 0.25 μmol). Curve I represents the linear response which is sometimes observed but curve II represents the more usual response of activity to enzyme concentration.

Table 1. Requirements for *sn*-glycerol-3-phosphate acyltransferase activity

Omissions	*sn*-Glycerol-3-phosphate acylated* (nmol)
Complete system	136
ATP	0.5
CoA	2.5
ATP and CoA	1
$MgCl_2/MnCl_2$	142
Palmitate	11
All of the above	2

* The assay of acyltransferase activity was carried out as described in Experimental using *sn*-glycerol-3-phosphate (0.1 μCi; 0.25 μmol). The incubation period was 60 min.

The dependence of the acyltransferase reaction on the addition of CoA, ATP and palmitic acid (Table 1) indicates that the palmitic acid has first to be converted to palmitoyl CoA. The assay described in Experimental assumes that the palmitoyl CoA generating system is non-limiting and, that this is so, is suggested by the fact that the addition of palmitoyl CoA did not further enhance incorporation of [U-^{14}C]-*sn*-glycerol-3-phosphate into lipids. More direct assay of the fatty acyl-CoA synthetases was not possible (see section on Substrate specificity of the acyltransferase system). Optimum concentrations of these compounds and of the second (labelled) substrate, *sn*-glycerol-3-

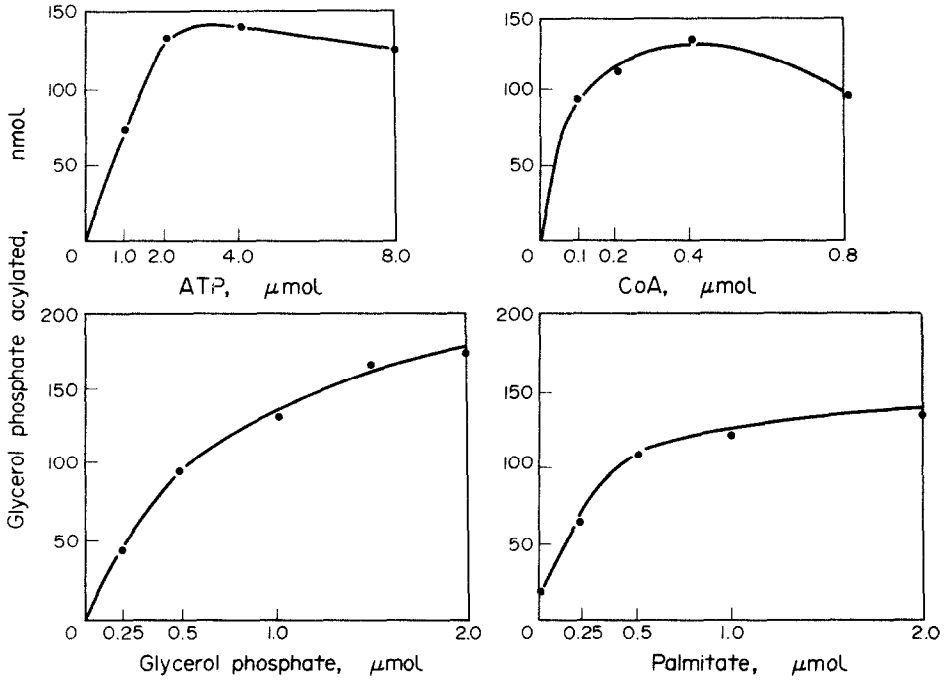

Fig. 2. The effect of cofactor and substrate concentrations on acyltransferase activity. Reaction mixtures containing filtered homogenate (35 mg of protein) were incubated for 60 min and the labelled products determined as described in Experimental using *sn*-glycerol-3-phosphate (0.1 μCi; 0.25 μmol).

phosphate, for acyltransferase activity were determined (see Fig. 2). For reasons of economy the highest concentration of the labelled substrate, *sn*-glycerol-3-phosphate, used in the substrate concentration curve (Fig. 2) was 2 μmol per assay of 1.3 ml and even this amount of substrate did not completely saturate the reaction. Similarly, some experiments were carried out with *sn*-glycerol-3-phosphate (0.1 μCi; 0.25 μmol) per assay; however, in many experiments, specified in the text, the concentration was increased to 1.5 μmol and 2.5 μCi per assay. The optimum pH for the reaction was 7.9–8.

Identification of products of the reaction

Two dimensional-TLC on Si gel, employing the solvent systems of Marinetti [10], was used to tentatively identify the labelled products of the reaction (see Experimental). The products were *lyso*phosphatidic acid, phosphatidic acid, mono-, di-, and tri-acylglycerols and contained 38.7, 14.5, 27.9, 13.2 and 5.4 % of the counts, respectively. The identities of the labelled products were further confirmed by testing for the presence or absence of phosphate groups with 5-sulphosalicyclic acid spray [11a]; endogenous phosphatidyl-serine, -ethanolamine and -choline, identified by comparison with markers and with either ninhydrin [11b] or Dragendorff reagent [11c] sprays, remained unlabelled.

Substrate specificity of the acyltransferase system

The fatty acid substrate specificity of the acyltransferase system was investigated using free fatty acids dissolved in ethanol. The results are shown in Table 2. Since the fatty acids are not especially soluble in dilute aqueous ethanol, it was thought the results might reflect the solubility of the various acids rather than the true specificity of the enzyme for these substrates. For this reason, experiments were carried out in the presence of 3, 10, 20 and 40 % (v/v) ethanol to increase the solubility of the palmitic acid. The rates of acylation of *sn*-glycerol-3-phosphate were 48, 16.5, 0 and 0 nmol per hr, respectively; thus, 20 % ethanol, in which the fatty acid (1.5 μmol) is not fully soluble, completely inhibits activity. Another approach to the problem was sought by repeating the substrate specificity experiments using the water-soluble polyoxyethylene sorbitan esters of fatty acids (Tweens) and also by using the more readily dispersable triethanolamine esters prepared as described in Experimental. Experiments 1 and 2 (Table 2) indicate that the specificity of the acyltransferase system is palmitate > stearate > oleate > linoleate > linolenate > laurate. Experiments 3, 4 and 5 (Table 2) indicate that the more readily soluble and dispersable polyoxyethylene sorbitan and triethanolamine esters are also active as substrates. As the activity in the presence of the free acid is generally equal to or greater than the activity in the presence of the esters, it appears that the solubility of the free fatty acids in dilute aqueous ethanol is sufficient to saturate the acyltransferase system. Consequently, in all subsequent experiments the free acid was used as substrate.

Table 2. Fatty acid substrate specificity of the acyltransferase system

Substrate	*sn*-Glycerol-3-phosphate acylated* (nmol per mg protein) Experiment No. 1	2	3	4	5
Palmitic acid	192	187	104		
Triethanolamine palmitate			82		
Tween 40†			106		
Stearic acid	180	135		130	
Triethanolamine stearate				118	
Oleic acid	110				204
Triethanolamine oleate					216
Tween 80†					103
Linoleic acid	59				
Triethanolamine linoleate	45				
Linolenic acid	49				
Triethanolamine linolenate	47				
Lauric acid	4	2.7			
Tween 20†		26.0			

* Acyltransferase activity was assayed as described in Experimental using *sn*-glycerol-3-phosphate (2.5 μCi; 1.5 μmol). The incubation period was 60 min.

† Tween 20, 40 and 80 are the polyoxyethylene sorbitan esters of laurate, palmitate and oleate, respectivelv.

It is possible that these results actually indicate the order of specificity of the fatty acyl-CoA synthetase for the various substrates. However, we were unable to demonstrate this enzyme using the hydroxamate assay of Tuttle and Lipman, and the turbidity, as well as the presence of UV-absorbing materials in the homogenate, made it impracticable to detect the formation of thioester linkages spectrophotometrically at 230 nm.

There seems to be little correlation between the order of rates of acyltransferase activity with the actual percentage distribution of various fatty acids in the endogenous lipid of the cotyledons 30 days after flowering (DAF), which, as found by Porra and Stumpf [12], contain a low concentration of stearate (1 %) and higher concentrations of linoleate (55 %), palmitate (20 %), oleate (14 %), and linolenate (10 %). Shine *et al.* [13] have suggested that low stearyl-ACP thioesterase activity coupled with high stearyl-ACP desaturase and oleoyl-ACP thioesterase accounts for the low concentrations of stearate in the lipids of many plants. If their suggestion is correct there would be little free stearic acid available for the relatively active system observed in this paper for the acylation of *sn*-glycerol-3-phosphate by free stearic acid, thus explaining the apparent anomaly. The reason for the high concentration of linoleate and lower concentration of oleate in soybean lipid, despite a relatively low rate of acylation of linoleate as compared to oleate (Table 2), might also be explained by the biosynthetic sequence postulated by Shine *et al.* [13] by assuming that the rate of desaturation of oleoyl CoA to linoleoyl CoA in the *in vivo* situation is greater than the rate of acylation of oleoyl CoA. In this way, more linoleoyl CoA than oleoyl CoA could be available to the acyltransferase system. Further, the rate of acylation of linoleic acid (Table 2) may be limited by the rate of the prior conversion of linoleic acid to linoleoyl CoA which is required under assay conditions.

Yet another explanation for the high linoleate content of storage oils in plant seeds is as follows. It is generally agreed [14, 15] that oleate in oil seeds arises from the desaturation of stearyl ACP to oleoyl ACP. However, it has been proposed that in micro-organisms [16, 17] oleate is converted to linoleate, and presumably further to linolenate, not by a sequential desaturation of oleoyl CoA to linoleoyl CoA and linolenyl CoA as suggested by Shine *et al.* [13], but by desaturation of 2-oleoylphosphatidylcholine to 2-linoleoylphosphatidylcholine and

Table 3. Distribution of acyltransferase activity in soybean cotyledon cells

Entry	Fraction	Acyltransferase activity* (nmol *sn*-glycerol-3-phosphate acylated per ml† per hr) Expt. 1‡	Expt. 2§	Expt. 3§
1.	Whole homogenate	301.0	150.0	280.0
2.		4.6	3.9	3.1
3.	10000 *g* Pellet + cytosol	44.3	29.9	31.1
4.	40000 *g* Pellet	5.0	0.6	1.2
5.	40000 *g* Pellet + cytosol	38.7	4.8	4.1
6.	150000 *g* Pellet	2.3	1.8	1.6
7.	150000 *g* Pellet + cytosol	61.0	6.6	11.2
8.	Lipid layer	2.0	1.4	7.2
9.	Lipid layer + cytosol	47.8	64.9	27.2
10.	Cytosol	41.4	2.5	2.6
11.	10000 *g* Pellet + lipid layer + cytosol	—	—	58.6
12.	Addition of entries 3, 5, 7, 9 and 10	233.0	109.0	76.2
13.	Entry 12 minus 4 × (entry 10)	67.6	98.7	65.8

* Acyltransferase activity was assayed as described in Experimental using *sn*-glycerol-3-phosphate (2.5 μCi; 1.5 μmol). Incubation time, 60 min.

† All preparations were made up to a final volume equal to the volume of filtered homogenate from which they were derived.

‡ The filtered homogenate, from which the fractions were derived, was prepared using an Ultra-Turrax homogenizer as described in Experimental.

§ The fractions were prepared as described in Experimental except that the filtered homogenate was prepared using the more gentle cell-disrupting technique.

2-linolenylphosphatidylcholine. Thus, the oleate is already acylated prior to desaturation. If this latter pathway is the preferred pathway for the formation of polyunsaturated fatty acids, then the rates of reaction of the acyltransferase system for linoleate and linolenate become irrelevant and the proportion of oleate, linoleate and linolenate will reflect the relative activities of the following enzymes systems: the system converting stearyl ACP to oleoyl CoA via oleoyl ACP; the acylation of oleoyl CoA; and, the desaturases requiring phosphatidylcholine derivatives of oleic and linoleic acids as substrates. It is interesting to note that Slack *et al.* [18] have presented labelling experiments which support a similar role in oil seed cotyledons for phosphatidylcholines both in the formation of polyunsaturated fatty acids and as diacylglycerol donors for the synthesis of other lipids. Further, the same authors presented evidence suggesting that triacylglycerols are formed via phosphatidylcholine rather than phosphatidic acid: however, in the experiments described here little or no label was found associated with choline-containing lipids.

Sub-cellular location of acyltransferase activity

As stated earlier, the acyltransferase system in soybean homogenates contains *ca* 6 enzymes and it would not be surprising, therefore, if these enzymes did not all reside at the same location within the cell. Furthermore, phospholipid-rich membranes are found in all organelles throughout the cell: for this reason again, it would not be surprising if acyltransferase could be demonstrated at many sites within the same cell. Indeed there have been many reports in the literature confirming that acyltransferase systems do have a complex distribution pattern in a great variety of cells. In animal [2] and yeast [3] cells, acyltransferase activity has been demonstrated in mitochondria and microsomes. In *Euglena gracilis*, acyltransferases have been reported in chloroplasts, mitochondria and microsomes [20]. In higher plants, the activity has been located in chloroplasts, mitochondria, microsomes and 'fat bodies' [7, 21].

The results in Table 3 show that acyltransferases of soybean cotyledons also have a complex intra-cellular distribution. When the various sub-cellular fractions were obtained using an Ultra-Turrax homogenizer as described in the Experimental, the particulate fractions alone had very little acyltransferase activity but the cytosol was very active; furthermore, the addition of cytosol to the various particulate fractions did not lead to any significant stimulation of cytosol activity (Expt. 1; Table 3). When the fractions were derived from a homogenate prepared by the gentler chopping technique of Spencer and Wildman [19], a different pattern of results emerged (Expts. 2 and 3; Table 3). Now the cytosol, as well as the particulate fractions, showed little activity but there was a marked stimulation of activity when the 10000 *g* pellet or lipid layer fractions were assayed in the presence of cytosol, suggesting that the cytosol contains enzymes or cofactors which are either not present in these particulate fractions or present only in limiting amounts.

Taken together, these results suggest that both the organelles of the 10000 *g* pellet, presumably plastids, mitochondria and glyoxysomes [22], and of the lipid layer, presumably 'fat bodies' [6, 7], are damaged when the homogenates are prepared with the Ultra-Turrax homogenizer releasing enzymes into the cytosol. Further confirmation of this view was obtained by comparing entries 12 and 13 of Table 3. Entry 12 is the sum of entries 3, 5, 7, 9 and 10, but since each of these 5 entries in the addition contained a contribution for cytosol, a further entry, entry 13, was compiled by subtracting from entry 12 the value for cytosol (entry 10) multiplied by 4. Only in Expt. 1 (Table 3), where a harsh cell-disruption technique was used, was entry 13 considerably less than entry 12, suggesting that the cytosol contains most of the enzymes of the acyltransferase system as a result of leakage from damaged organelles. Where gentler techniques of cell breakage were employed (Expts. 2 and 3;

Table 4. Effect of seed development and germination on cotyledon acyltransferase system activity

Period of seed development or germination	Acyltransferase system activity* Expressed as nmol of *sn*-glycerol-3-phosphate acylated per g (fr. wt) per hr		Expressed as nmol of *sn*-glycerol-3-phosphate acylated per mg protein per hr
	Expt. 1	Expt. 2	Expt. 2
15 DAF†	112	265	23.5
20 DAF	172	305	17.5
30 DAF	652	133	14.5
40 DAF	1240	1270	13.2
45 DAF	998	1000	9.2
	Expt. 3		Expt. 3
2.5 DAG‡	616		44.8
5.5 DAG	123		24.6

* Acyltransferase activity was measured as described in Experimental using *sn*-glycerol-3-phosphate (2.5 μCi; 1.5 μmol). Incubation period, 20 min.
† DAF: Days after flowering.
‡ DAG: Days after germination.

Table 3), entry 13 was only slightly lower than entry 12.

Comparing entry 13 with entry 1 shows that the recovery of total activity of the various sub-cellular fractions was 22.5, 65.6 and 23.5% of that observed in the homogenate in Expts. 1, 2 and 3, respectively. It is surprising that no activity was detected in the microsomal pellets since acyltransferase activity has been found in the endoplasmic reticulum fraction of castor bean endosperm [23].

Effect of seed development and germination on cotyledon acyltransferase

Because of the failure to obtain a linear response between the activity of the acyltransferase complex activity and protein concentration (Fig. 1), it was difficult to determine the effect of cotyledon development and seed germination on acyltransferase activity. It was decided that acyltransferase activity would have to be determined in two ways. Firstly by assaying aliquots of the various homogenates so that there was an identical protein content (12 mg) in each case: in such assays the results were expressed as nmol of *sn*-glycerol-3-phosphate acylated per mg of protein per hr (Table 4). Secondly, the enzyme was assayed by using aliquots of the various homogenates representing an identical fr. wt (0.5 g): in these assays the results were expressed as nmol of *sn*-glycerol-3-phosphate acylated per g fr. wt of cotyledon per hr (Table 4).

The results show that during cotyledon development the activity of the acyltransferase complex increased for 40 DAF, when measured on a fr. wt basis, but then decreased slightly during the next 5 days. This increase in enzyme activity closely parallels the observed increase in lipid content of the cotyledon per g fr. wt as measured by Porra and Stumpf [12]; the lipid content increased to a maximum at 40 DAF and remained steady for the following 10 days. There was a decrease in activity on the basis of protein throughout the entire period of 45 days. This indicates that for at least the first 40 DAF there is either synthesis or activation of the enzymes involved, and that the seed content of these enzymes increases during this period but then declines slightly during the next 5 days. However, the results also indicate that during cotyledon development there is an even greater synthesis of proteins other than these enzymes, so that there is a decrease in, or dilution of activity per unit wt of protein. The proteins which cause this 'dilution' effect are probably legume storage proteins.

In the germination experiments, there is a decrease in acyltransferase activity when measured on a fr. wt or protein basis suggesting that the enzymes are not being synthesized at a rate sufficient to compensate for turnover. The fact that the specific activity, at 2.5 days after germination (DAG), of 44.8 nmol of *sn*-glycerol-3-phosphate acylated per mg of protein per hr is higher than at 45 DAF (i.e. 9.2) suggests that the storage protein is degraded more rapidly than the acyltransferase system.

In previous papers in which the conversion of acetate and malonate to fatty acids [12] and the desaturation of stearyl ACP to oleoyl ACP [15] were considered, it was found that fatty acid synthesis by pea cotyledon homogenates from ^{14}C-labelled malonate, measured by the assay of Porra and Stumpf [12], was 0.81 nmol of malonate incorporated per hr per mg of protein and was higher than in soybean homogenates (0.19); nonetheless, soybean cotyledons are richer in storage oil (70 mg per g fr. wt) than pea cotyledons (14.5). Thus, if there is a single universal mechanism controlling oil formation which is common to both pea and soybean cotyledons, then it would seem that the rate-limiting and regulating step of triacylglycerol formation is not located in the biosynthesis of fatty acids, but may be associated with the formation of the glycerol moiety or with the fatty acid esterification of this moiety; indeed, this was the reason for undertaking the studies described here. The results in Table 4, showing an increase in the activity of *sn*-glycerol-3-phosphate acyltransferase during cotyledon development and concomitant lipid biosynthesis, suggest that the acyltransferase system may have a regulatory role in triacylglycerol formation. Because of the multi-enzyme nature of the acyltransferase system, as measured in this paper, it is not possible at this stage to specify if the increase in acyltransferase activity is due to the synthesis of one specific component enzyme.

EXPERIMENTAL

Chemicals and materials. Tween 40 and Tween 80 (polyoxyethylene sorbitan-monopalmitate and -monooleate, respectively), DL-α-glycerophosphate, palmitoyl CoA, ATP and CoA

were supplied by Sigma. Stearic and oleic acids were supplied by BDH; lauric, palmitic, linoleic and linolenic acids, and triethanolamine were supplied by Fluka A.G. Triethanolamine esters of fatty acids were prepared by mixing equimolar quantities of triethanolamine and fatty acid. L-[U-^{14}C]-glycerol-3-phosphate was supplied by the Radiochemical Centre, Amersham, U.K.

Soybean cotyledon preparations. Grant strain soybean plants (*Glycine max* cv Merr.) were grown, the beans harvested and homogenized as previously described [12]. After filtration through Miracloth, the filtrate was centrifuged at 1000 *g* for 30 min and the supernatant again passed through Miracloth; the filtrate was designated filtered homogenate. Filtered homogenates prepared from beans harvested at 15, 20, 30, 40 and 45 DAF contained *ca* 17, 26, 35, 55 and 65 mg of protein per ml, respectively, using the procedure of ref. [24] with BSA as standard. Unless otherwise stated, the filtered homogenates used were prepared from 30 DAF beans.

Soybean cotyledon sub-cellular fractions. Whole homogenate, 10000, 40000 and 150000 *g* pellets were prepared as described in ref. [22]; in addition, a lipid layer was obtained by recovering the lipid floating upon the cytosol after centrifugation at 150000 *g*. The pellets and the lipid layer were resuspended and made to a final vol. equivalent to that of the whole homogenate from which they were derived.

Assay of sn-*glycerol-3-phosphate acyltransferase.* The assay used was similar to that of ref. [25]. The prepn to be assayed was incubated at 28° with (μmol): CoA (0.2), ATP (4), $MgCl_2$ (1), $MnCl_2$ (1), palmitic acid (in 50 mM EtOH; 2), [U-^{14}C]-*sn*-glycerol-3-phosphate (0.1 μCi; 0.25) and Tris–HCl buffer (pH 8.5; 5) in a final vol. of 1.3 ml. The labelled glycerol phosphate used in these assays was supplied as L-[U-^{14}C]-glycerol-3-phosphate while the unlabelled compound was supplied as DL-α-glycerolphosphate but the concns above indicate the concn of the natural substrate, *sn*-glycerol-3-phosphate. To terminate the reaction, MeOH (2 ml), $CHCl_3$ (4 ml) and 0.2 N HCl (1 ml) were added and the tube shaken vigorously. The bottom $CHCl_3$ layer was removed and washed × 3 with 4 ml 50% MeOH in 0.1 N HCl. The $CHCl_3$ layer was evapd to dryness under a stream of N_2, the residue redissolved in $CHCl_3$ (1 ml) and an aliquot (0.1 ml) placed on a glass paper disc in a counting vial. The $CHCl_3$ was removed by evapn before adding 7 ml 0.6% PPO (2,5-diphenyloxazole) in sulphur-free toluene. The vial was then counted by liquid scintillation with an efficiency of *ca* 75% for ^{14}C. Under these conditions, an incorporation of 2660 cpm per total assay mixture corresponded to an acylation of 10 nmol of *sn*-glycerol-3-phosphate. In certain expts, specified in the text, the sp. act. of the [U-^{14}C]-*sn*-glycerol-3-phosphate was increased to 2.5 μCi in 1.5 μmol: in this case an incorporation of 11100 cpm corresponded to the acylation of 10 nmol of *sn*-glycerol-3-phosphate.

Identification of labelled products of the reaction by 2-D TLC. Si gel films (No. 5748; E. Merck) were heated for 20 min at 95° prior to use. The labelled products, obtained in $CHCl_3$ soln as described above, were spotted onto the chromatogram. The solvent systems employed were *n*-heptane–di-*iso*butylketone–HOAc (85:15:1) in the first dimension and di-*iso*butylketone–HOAc–H_2O (40:25:1) in the second dimension [10]. Lipid spots were visualized using 2,6-dichlorofluorescein (0.1% in EtOH) and these spots were tentatively identified by comparison with authentic markers. Using a radiochromatogram scanner it was found that 5 of these spots were radioisotopically labelled (see Results and Discussion). The proportion of counts associated with each spot was calculated by determining the area under each peak.

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