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OIL BODIES AND LIPID SYNTHESIS IN DEVELOPING SOYBEAN SEEDS

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Abstract—The chemical composition of the oil bodies of soybean changed during seed development, proteins and phospholipids decreasing and polar lipids increasing thereby affecting to the density of the organelle (1.08–0.98 g/cm^3). As a consequence, the density of the isolation medium was adjusted to make the oil bodies float. The distribution of newly synthesized lipids in the different subcellular fractions showed the important role of the oil bodies in the general scheme of lipid synthesis in the seed. However, experiments using short incubation times indicated the preferential implication of the microsomal fraction in triacylglycerol synthesis. The hypothesis of an integrated structure of triacylglycerol synthesis and accumulation in the seed is discussed.

INTRODUCTION

Subcellular organelles known as oil bodies, oleosomes or reserve oil droplets, are the sites of triacylglycerol storage in the specialized cells of oily seeds and fruits. There is controversy about their origin, structure, function and even nomenclature. This debate arises from the variability in the chemical composition, ultrastructure and enzymatic activities of oil containing organelles from different plant tissues [1]. Thus oil bodies have been suggested to be different from spherosomes which are present in the vegetative cells of higher plants [2]. All oil containing organelles are formed essentially of lipids and proteins [1], triacylglycerols being the main components of the lipid fraction followed by phospholipids. This composition justifies the existence of a surrounding membrane as observed by ultrastructural studies. In a number of seeds the protein and phospholipid content is appropriate for the provision of a half-unit membrane [3,4]. However, in oil bodies from some other seeds there are excess proteins and phospholipids [5, 6].

Isolated oil bodies have been reported to synthesize neutral and polar lipids from malonyl-CoA or long chain acyl-CoA [6–9] the glycerol phosphate being accepted to be the major route for triacylglycerol synthesis. Evidence has also been provided for the presence of acyl-CoA synthetase and acyltransferase activities in these organelles [10]. On the other hand, the microsomal fraction has also been shown to catalyse the transfer of fatty acids from acylthioesters to polar lipids and triacylglycerols [11], as well as the acylation of glycerol-3-phosphate to give phosphatidic acid which is in turn converted to triacylglycerols via diacylglycerols [12]. A particulate fraction sedimenting at 1000 *g* has also been reported to be the main site of triacylglycerol synthesis [13]. It has been suggested that nascent oil bodies are formed near the surface of plastids being coated by a membrane synthesized from materials provided by the endoplasmic reticulum (ER) [4,13]. However, the polypeptide patterns of oil bodies and ER membranes are different [14–16]. Recently, a model system from safflower microsomes has been shown to synthesize triacylglycerols in sufficient quantity to demonstrate visually, oil deposition *in vitro* [17], the oil droplets being released into the medium as naked oil bodies. It has been also suggested that the oil bodies are formed in a 'protein reticulum' [18]. However, in spite of all the information available, the question still remains of how triacylglycerols are synthesized and stored *in vivo*.

Oil bodies are obtained as a floating layer after centrifugation of homogenized oil seeds. Previous results from this laboratory [19] showed that very young developing seeds lacked any floating layer after homogenization and centrifugation in 0.25–0.6 M sucrose or 0.4 M sorbitol. Nevertheless, when a denser medium was used, a floating layer was obtained independent of the age of the seeds.

In this paper a procedure to prepare oil bodies from developing soybean seeds is reported. Changes in the chemical composition of these organelles and the role of the different subcellular fractions in triacylglycerol synthesis and accumulation are also presented and discussed.

RESULTS AND DISCUSSION

Chemical composition of oil bodies

Oil bodies from soybean seeds at different stages of development were isolated and their chemical composition determined (Table 1). The main components were proteins, neutral lipids and phospholipids; glycolipids were absent. These results are in agreement with the reported composition of oil bodies from other seeds [6, 10]. The neutral lipid fraction contained triacylglycerols (90–95%), diacylglycerols (1–2.5%), free fatty acids (1–5%) and sterols (0.3–1%). The phospholipid fraction contained phosphatidylcholine (45–52%), phosphatidylethanolamine (20–25%) and phosphatidylinositol (25–30%) . The amino acid composition of the protein fraction was: Glu-Gln (18.5%), Asp-Asn (12.6%), Ser

Table 1. Chemical composition and calculated density of oil bodies from developing soybean seeds

Age of seeds (DAF)†	Protein (%)	Neutral lipids (%)	Phospholipids (%)	Others* (%)	Calculated density (g/cm^3)
20	32.0	46.6	20.3	1.0	1.08
22	25.1	58.6	14.7	1.6	1.04
24	22.3	69.4	5.0	3.3	1.01
26	22.1	69.8	5.8	2.3	1.01
33	21.9	70.0	6.4	1.7	1.01
40	14.6	81.1	3.4	0.8	0.98
42	15.2	79.8	4.6	0.4	0.98

* Pigments and other polar lipids.
† Days after flowering.

(9.8%), Gly (9.7%), Leu (8.4%), Ala (7.8%), Arg (5.7%), Thr (5.0%), Ile (4.8%), Lys (4.7%), Phe (4.1%), Val (3.1%), Tyr (2.6%), His (2.4%) and Met (0.7%).

During seed development the protein and phospholipid content decreased and that of neutral lipids increased, thereby affecting the density of the organelle. The density of the oil bodies was calculated from the densities of the proteins, phospholipids and neutral lipids fractions (1.41, 1.11 and 0.92 g/cm^3 respectively). The calculated density of the oil bodies from very young seeds was as high as 1.08 g/cm^3 but decreased during seed development and became 0.98 g/cm^3 in seeds sampled more than 40 days after flowering (DAF). The behaviour of the oil bodies in different isolation media was in agreement with the calculated densities. According to this, when 32% sucrose (1.134 g/cm^3) was used the oil bodies formed a floating layer independent of the age of seeds. However, when resuspended in 0.4 M sorbitol (1.034 g/cm^3) and centrifuged again, the oil bodies from very young seeds (less than 22 DAF) sedimented while those from more developed ones floated. In this way heavy and light oil bodies could be separated. At each developmental stage only one type of oil body was normally found but occasionally both types were present. It is interesting to note that grinding media which are normally used to isolated these organelles [6–9, 18] would not be sufficiently dense to make all the oil bodies float. When the protein and phospholipid contents make the density of the oil bodies higher than that of the isolation medium a partial precipitation of these organelles may occur.

In vivo *incorporation of* [2-^{14}C] *acetate*

Developing soybean seeds actively synthesized neutral and polar lipids as determined by incorporation of radioactive acetate (Table 2).The period of maximum activity was from 19 to 41 DAF, the synthesis of triacylglycerols being especially active during this period. Between 42 and 50 DAF the seeds were still active but the synthesis of polar lipids and especially triacylglycerols was reduced while the synthesis of diacylglycerols was maintained. Before 19 DAF and after 50 DAF, lipid synthesis proceeded much more slowly. The incorporation of acetate into the lipids of different subcellular fractions after the developing seeds were incubated with radioactive acetate for 15 hr at 25 is shown in Table 3. Under these conditions the oil bodies were more strongly labelled than any other fraction. The newly synthesized triacylglycerols, diacylglicerols and even polar lipids were found preferentially in the oil bodies, the triacylglicerols being almost exclusively in this fraction (92% of total). These results suggest that oil bodies play an important role not only in triacylglycerol accumulation but also in the general scheme of lipid synthesis in the seeds. In order to clarify this role and the possible cooperation among the different subcellular fractions in triacylglycerol synthesis and accumulation, further experiments were carried out. In these incubation times were reduced to minimize the effect of lipid accumulation (Table 4). In this way, the incorporation into lipids of all fractions increased with incubation time. In all cases oil bodies were more strongly

Table 2 Incorporation of [2-^{14}C] acetate into lipids of developing soybean seeds after 4 hr incubation.

Age of seeds (DAF)*	nmol of acetate incorporated into: Total lipids	Triacylglycerols	Diacylglycerols	Polar lipids
15–17	34.0 ± 1.9	2.9 ± 0.1	4.9 ± 0.4	26.2 ± 1.5
19–41	78.4 ± 8.8	16.7 ± 3.4	10.7 ± 3.0	50.9 ± 6.6
42–50	63.3 ± 6.0	8.9 ± 1.5	12.3 ± 0.9	42.0 ± 5.2
52–64	29.1 ± 6.7	2.6 ± 0.7	7.3 ± 1.9	19.2 ± 4.8

Results are expressed as mean ± s.d.
* Days after flowering.

Table 3. Incorporation of [2-^{14}C] acetate into lipids of different subcellular fractions prepared from developing soybean seeds after 15 hr of incubation *in vivo*

	nmol of acetate incorporated into:				
Subcellular fraction	Total lipids	Triacyl-glycerols	Free fatty acids	Diacyl-glycerols	Polar lipids
Oil bodies	62.1	26.6	1.3	8.0	23.2
Microsomes	18.5	1.4	0.4	1.8	14.7
Precipitate	7.0	1.0	0.3	1.0	4.5
Supernatant	3.4	0.0	0.4	0.3	2.7

labelled than the other fractions, but contained little triacylglycerols, the main components being polar lipids. The microsomal fraction contained triacylglycerols in highly variable amounts; however, triacylglycerols always represented higher percentages (20–30%) of labelled lipids as compared with oil bodies (9–15%). Polar lipids and diacylglycerols were also present in this fraction. The precipitate and the supernatant contained significant amounts of all lipids, including triacylglycerols. Although in some experiments the incorporation of acetate was almost negligible, when incubated for < 5 sec, in three of them a high lipid synthesis capacity was observed (Table 5). In addition, these seeds were also very active in triacylglycerol synthesis compared with those of Table 4. The incorporation took place preferently into the microsomal lipids (Table 5). Interestingly microsomes contained *ca* 90% of the newly synthesized triacylglycerols, whereas oil bodies contained very little. The existence of two types of seeds which incorporated very different amounts of acetate cannot be explained with the present results. However, the distribution of the newly synthesized fatty acids in the different lipids (data not shown) suggested that the mechanism of triacylglycerol synthesis operated in a different way in both types of seeds. The study of the fatty acid distribution as well as the exchange of fatty acids and glycerolipids among the different subcellular fractions is under progress and will be the matter of a future paper.

In vitro *incorporation of* [1-^{14}C] *oleate*

To check lipid synthesizing capacity isolated fractions were incubated with [1-^{14}C] oleoyl-CoA (Table 6). In all

Table 4. Incorporation of [2-^{14}C] acetate into lipids of different subcellular fractions prepared from developing soybean seeds incubated *in vivo*

		pmol of acetate incorporated into:				
Time of incubation	Subcellular fraction	Total lipids	Triacyl-glycerols	Free fatty acids	Diacyl-glycerols	Polar lipids
	Oil bodies	7.4± 1.3	1.1± 0.3	1.6±0.5	1.4± 0.6	3.0± 1.1
< 5 sec	Microsomes	2.3± 1.0	0.4±0.2	0.4±0.3	0.7± 0.4	0.7± 0.1
	Precipitate	4.7± 0.7	0.6± 0.2	1.2±0.3	0.7± 0.2	1.9± 0.1
	Oil bodies	42.3±10.6	4.0± 0.6	5.4±0.4	8.9± 3.1	22.5± 6.5
1 min	Microsomes	10.4± 5.7	2.0± 1.5	1.4±0.9	2.2± 1.7	4.3± 1.5
	Precipitate	20.5± 8.1	2.5± 1.3	5.2±2.7	2.5± 0.8	7.7± 4.6
	Supernatant	5.7± 1.1	1.0± 0.3	1.9±0.3	1.0± 0.1	1.1± 0.5
	Oil bodies	287.0±17.7	34.7± 3.3	21.6±5.3	53.7±11.6	166.7±20.0
10 min	Microsomes	60.7±21.7	18.7±16.3	4.7±1.3	10.3± 4.0	24.0±10.3
	Precipitate	85.3±13.7	9.7± 2.3	8.7±2.7	13.3± 5.3	51.3± 4.3
	Supernatant	25.3± 6.7	2.0± 0.7	5.3±1.7	3.7± 1.3	12.7± 4.3

Results are expressed as mean ± s.d. (4 experiments).

Table 5. Incorporation of [2-^{14}C] acetate into lipids of subcellular fractions prepared from developing soybean seeds* after a very short incubation time (< 5 sec) *in vivo*

	pmol of acetate incorporated into:				
Subcellular fraction	Total lipids	Triacyl-glycerols	Free fatty acids	Diacyl-glycerols	Polar lipids
Oil bodies	15.2±13.7	3.6± 3.0	1.4±0.7	6.4±6.9	2.8±2.6
Microsomes	61.6±15.1	38.9±11.0	4.6±2.9	9.4±1.5	7.1±2.0
Precipitate	4.3± 2.2	1.3± 0.6	0.6±0.2	0.6±0.4	1.6±1.5

Results are expressed as mean ± s.d. (3 experiments).
*Seeds of high triacylglycerols synthesis capacity.

Table 6. Metabolism of [1-^{14}C] oleoyl-CoA in different subcellular fractions from developing soybean seeds

Subcellular fraction	pmol of oleate incorporated into: Acyl-CoA	Triacyl-glycerols	Free fatty acids	Diacyl-glycerols	Polar lipids
Total homogenate	240	180	540	110	1310
Boiled oil bodies	2380	0	0	0	0
Oil bodies	230	90	300	40	1710
Microsomes	380	100	620	150	1130
Precipitate	1050	30	220	90	990
Supernatant	160	10	1770	80	350

fractions oleate was transferred to the different lipid classes. Thioesterase activity was detected in all fractions, especially in the supernatant. Polar lipids were actively synthesized in all fractions but microsomes were the most active in synthesizing di- and triacylglycerols. Nevertheless, oil bodies and the precipitate were also active.

The results presented here suggest that components of the microsomal fraction are the primary sites of triacylglycerol synthesis in soybean seeds. However, the rapid accumulation of the newly synthesized triacylglycerols in the oil bodies *in vivo*, in accordance with the results of an *in vitro* system [17], and the presence of the enzymatic activities of lipid synthesis in the oil bodies (cf. 6, 11–14) support the existence of an integrated structure where triacylglycerols are synthesized and accumulated. This would correspond to the model proposed by Wanner *et al.* [4] in which plastids and ER are involved in triacylglycerol synthesis and oil body production. This structure would be destroyed during homogenization and its components recovered as oil bodies and other particulate fractions. The distribution of enzymatic activities of lipid synthesis in the different subcellular fractions, in both *in vivo* and *in vitro* experiments, is in agreement with this hypothesis.

EXPERIMENTAL

Developing seeds were obtained from *Glycine max* L. (cv Williams) plants grown soil-free using a 12 hr photoperiod (9600 l ×) at 22–24° and 12 hr night at 16–18°. To prepare subcellular fractions 5 g of seeds were homogenized in 32% sucrose, 50 mM Tris, pH 7.5 and fractionated by centrifugation at 20 000 *g* into floating layer (oil bodies), ppt. and supernatant. Microsomes were obtained from this supernatant [20]. All particulate fractions were resuspended in 0.4 M sorbitol, 0.1 M K-Pi, pH 7.5 and centrifuged again and the operation repeated × 3.

Chemical composition of oil bodies. Portions (3–5) of the washed floating layer were placed into test tubes. The lipids were extd × 3 with petrol–*i*PrOH (3:2) [21], evapd and weighed. The remaining protein was detmd in the same tube [22]. Lipids from portions were mixed and fractionated by silica gel CC [23] into neutral lipids, glycolipids and phospholipids. Lipid classes of these fractions were sepd by TLC on silica gel developed with petrol–Et_2O–HCO_2H (75:25:1) and quantified by GC of the corresponding Me esters after adding heptadecanoic acid as the int standard. The amino acid composition was determined in the protein fraction after hydrolysis with 6 M HCl at 110° for 18 hr. Amino acids were sepd by LC on Aminex 9-A (Bio-Rad) and determined fluorimetrically [24]. The density of the protein was calculated from the amino acid composition [25]. The density of the neutral and polar lipids was considered to be that of refined soybean oil and total phospholipids from soybean and were determined using a pycnometer.

In vivo incubations. Capacities for lipid synthesis were determined by incubating 0.3 g of sliced seeds in 0.6 ml of [2-^{14}C] acetate soln (148 KBq; 2.1 GBq/mmol, Amersham) at 25° for 4 hr. To study the incorporation into lipids of subcellular fractions, 1 g of sliced seeds (27–37 DAF) was incubated with [2-^{14}C] acetate (1.83 MBq)at 25° for the period of time indicated in the legend of the Tables. The tissue was then homogenized and fractionated as described above.

In vitro incubations. Isolated subcellular fractions corresponding to 1 g of seeds were incubated with [1-^{14}C] oleoyl-CoA (5 MBq; 2.1 GBq/mmol, Amersham) for 2 hr at 25°. After incubation the acyl-CoA and lipids were sepd [26], fractionated by TLC and assayed for radioactivity after adding 10 ml toluene–PCS (Amersham).

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