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Lipid Biosynthesis and Composition in Oil Bodies and Microsomes of Olive Fruit

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Both lipid synthesis and composition in oil bodies and microsomes of olive fruit at the first stage of development have been studied. The rate of fatty-acid synthesis in isolated oil bodies was saturated by 4.0 μM [2-^{14}C]-malonyl-CoA. The fatty-acids synthesized of phospholipids and neutral lipids were saturated and monounsaturated. Neutral lipids, galactolipids and, above all, phospholipids were the major acyl-lipid components of microsomal fraction, oleic and palmitic being their principal fatty-acids. When the lipids of microsomes were labelled *in vivo* with [1-^{14}C]-acetate, phospholipids and neutral lipids exibited a higher biosynthesis rate relative to the galactolipids. The increase in saturated and monounsaturated fatty-acid synthesis in microsomes, was also accompanied by an important [1-^{14}C]-acetate incorporation into polyunsaturated acids. The data presented here, in conjunction with our previous morphological results, suggest the possibility that olive fruit oil bodies could contain the necessary enzymes for the reserve lipid biosynthesis.

Key words: *Olea europaea*, Oil bodies, Microsomes, Lipid biosynthesis.

The study on reserve lipid accumulation in oleaginous fruit and seeds was begun with the works of DRENNAN and CANVIN (8) and HARWOOD and STUMPF (11), who detected in seeds cytoplasmic particles very similar to protoplastids which developed a high lipid synthesis capacity. These data together with the ultrastructural research (9, 16, 24) in relation to membranous particles located in the cytoplasm, led several researchers to suggest the existence of cellular organelles (proplastid and oil bodies) with a specific function relative to synthesis and accumulation of lipids (1, 2, 10). In this respect, POLLARD *et al.* (20) and MURPHY *et al.* (19) observed in tissues with a high oil content, that the proplastid is the source of the primary fatty-acids, and the endoplasmic reticulum is the site of further modifications where the desaturation and esterification of fatty-acids and the biosynthesis of phospholipids take place. Furthermore, investigations on microsome and oil body preparations from developing oil seeds indicate that acyl-CoA are the substrates for reactions occurring in the endoplasmic reticulum and oil bodies, and leading to the synthesis of triacylglycerols (13, 21). Thus, the problem

of ascertaining the intracellular location of the triacylglycerol synthesis to produce the final oil bodies is not firmaly settled, but there is evidence of a prominent role being played by the endoplasmic reticulum. From electron micrographs it appears that the membrane surrounding the oil body is formed by vesiculation of the endoplasmic reticulum with the triacylglycerol inserted between the two layers of the membrane of the endoplasmic reticulum (22).

In spite of its importance there is very little information about these organelles in oil fruits. In previous works, the chemical composition, the *in vivo* lipid biosynthesis and structural aspects of oil bodies in the mesocarp cells of olive fruit were studied (5, 7). Thus, before maturation, important morphological events take place in the cytoplasm, the most important one being the rapid differentiation of the lipid bodies and the subsequent reticulum endoplasmic cisterns which accompany oil bodies development.

From these results, it is possible to suggest the existence of a membranous expansion surrounding the oil bodies in olive fruit. In order to investigate the synthesis of storage triacylglycerols we have prepared oil body and microsome fractions from olive fruit, and we report the ability of these preparations to synthesize fatty-acids from ^{14}C-acetate.

Materials and Methods

Plant Material. — Fruits from olive trees (*Olea europaea L.*, cv. Marteño) were collected at 20-day intervals from June to August and the following steps were carried out.

Isolation of oil body and microsomal fractions. — Oil bodies were isolated and purified according to DAZA and DONAIRE (5). The microsomal pellets were also prepared from an extract of fruits on each day of the experiments. Fruits (50 g fresh weight) were homogenized for 2 min at 4°C with the aid of a mortar in a grinding medium containing 5 vols of 100 mM Tris-HCl buffer, pH 7.5, 400 mM sucrose, 1 mM $MgCl_2$, 10 mM KCl, 3 mM EDTA, 0.1% bovine serum albumin (BSA) and 1.5% insoluble polyvinylpyrrolidone (PVP). The homogenate was filtered and centrifuged at 10,000 × *g* for 20 min. The supernatant was again centrifuged at 105,000 × *g* for 60 min and the pellet suspended in 100 mM Tris-HCl, pH 7.5, containing 400 mM sucrose, 5 mM $MgCl_2$ and 2.5 mM dithiothreitol (DTT). The suspension was used as a microsomal fraction for lipid analysis and biosynthesis.

In vivo *labelling of microsome lipids.* — Aqueous microdroplets of [1-^{14}C]-acetate (specific activity 48 mCi/mmol) were put on 10 fruits (10 microliter of a 50 nCi/ml solution of acetate on each fruit), and the fruits were incubated in the conditions previously described (6). After 24 h the ^{14}C labelled lipids were analysed in microsomes isolated from labelled fruits.

In vivo *labelling of oil body lipids.* — Oil bodies isolated from fruits at first stage of development were incubated with 5 nmol of [2-^{14}C]malonyl-CoA (specific activity 53 nCi/nmol), as a fatty-acid precursor, in a medium containing 10 mM phosphate buffer, pH 7.2, 1 mM CoA, 1 mM NADPH, 0.1% BSA, 2 mM ATP, 1 mM DTT and oil body fraction (0.2 to 0.5 mg of protein) in a final volume of 0.1 ml. After incubation at 25°C for 30 min with constant shaking, total lipids were extracted from the reaction mixture and separated by thin layer chromatography. The fatty-acid methyl esters obtained from the total and individual lipids were assayed for ^{14}C as described in a previous work (6).

Analysis of lipids. — The BLIGH and DYER (3) method was used to extract

total lipids. To separate the different lipid fraction and fatty-acids we used thin layer chromatography following LEPAGE (12) and MORRIS *et al.* (18) methods. The methyl esters of the corresponding fatty-acids were prepared for gas chromatography analysis according to METCALFE *et al.* (15).

Results and Discussion

Figure 1 shows the optimal values corresponding to incubation time and concentration of protein and [2-^{14}C]malonyl-CoA of the incubation mixture, for ^{14}C incorporation into total fatty-acids of oil bodies isolated from olive fruit at the first stage of development. Initial experiments confirmed the requirement for NADPH, ATP and, above all, CoA as cofactors in fatty-acids synthesis assays (data not shown). The rate fatty-acid synthesis was more than 90% saturated by 4 μM malonyl-CoA. In this respect, GURR *et al.* (10) had already observed that the fat fraction isolated from immature seeds of *Crambe abyssinica* readily incorporated malonyl-CoA into fatty-acids. Saturated and monounsaturated fatty-acids of phospholipids and neutral lipids are the only fatty-acids synthesized by isolated olive fruit oil bodies (table I). The lack of polyunsaturated fatty-acids formation by isolated oil bodies is not in agreement with the *in vivo* [1-^{14}C]-acetate incorporation into oil body fatty-acids, where an important synthesis of linoleic acid was observed (4). This difference between *in vivo* and *in vitro* capacity, suggests the existence of an intracellular cooperation between oil bodies and other organelles for the oil body fatty-acid biosynthesis in olive fruit. These results have also been demonstrated in oil bodies from different oleaginous seeds. (11). In this respect, WEAIRE and KEKWICK (23) reported that plastid preparations obtained from homogenates of avocado mesocarp, incorporated ^{14}C-acetate into fatty-acids, principally palmitate and oleate, whereas the cytosolic protein fraction was essentially inactive. They concluded that the proplastids were the principal site of fatty-acid biosynthesis. Moreover, MOREAU and STUMPF (17) proved that the enzymes for desaturation of oleate and triacylglycerol synthesis were all localized in the endoplasmic reticulum membranes.

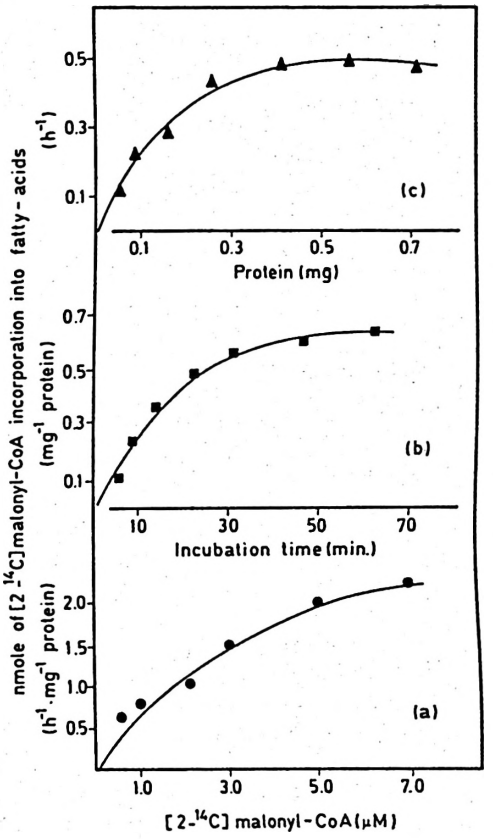

Fig. 1. *Effect of* [2-^{14}C]*malonyl-CoA concentration (a), incubation time (b) and protein concentration (c) on* ^{14}C *incorporation into total fatty-acids of oil bodies isolated from olive fruit at the first stage of development (fresh weight of 1 fruit, 0.3 to 0.5 g).* Mean of three independent assays are shown and the mean error was about 11% (a), 15% (b) and 17% (c).

Table II shows that neutral lipids, ga-

Table I. In vitro [2-^{14}C]*malonyl-CoA incorporation into fatty-acids of phospholipids and neutral lipids of oil bodies isolated from olive fruit at the first stage of fruit development (fresh weight of 1 fruit, 0.3 to 0.5 g).*
Results in percentage of total ^{14}C incorporated into fatty-acid of each lipid fraction. The mean of four different assays (± SD) are shown.

Fatty-acids	Phospholipids	Neutral lipids
Saturated	30.3 ± 4.1	14.3 ± 2.1
Monounsaturated	69.7 ± 7.8	85.7 ± 10.3

Table III. In vivo [*1-^{14}C*] *acetate incorporation into fatty-acids of total lipids, in microsomes of olive fruit at the first stage of development (fresh weight of 1 fruit, 0.3 to 0.5 g).*
Results, during each time interval, in percentage of total ^{14}C incorporated into fatty-acids. Mean of three independent assays (± SD) are shown.

Incubation time (h)	Saturated	Monoun-saturated	Polyun-saturated
1	18.3 ± 1.8	53.2 ± 6.9	28.5 ± 2.7
10	38.9 ± 4.4	51.0 ± 6.7	10.1 ± 1.1
24	42.1 ± 5.9	50.9 ± 5.8	7.0 ± 0.8

lactolipids and, above all, phospholipids are the major acyl-lipid components of microsomal fraction. The *in vivo* [1-^{14}C]-acetate incorporation into different acyl-lipids of olive fruit microsomes, and a greater phospholipid and neutral lipid biosynthesis relative to galactolipids are also observed in table II.

Although saturated and monounsaturated are the principal fatty-acids biosynthesized in microsomes, a high [1-^{14}]-acetate incorporation into polyunsaturated acids, above all at less than 10 h of incubation, is detected (table III).

Table II. *Acyl-lipid composition and* in vivo [*1-^{14}C*] *acetate incorporation into these compounds, in olive fruit microsomes at the first stage of fruit development (fresh weight of 1 fruit, 0.3 to 0.5 g).*
Results in percentage of acyl-lipids in total lipids and in percentage of ^{14}C incorporated in each lipid. Mean of three different assays (± SD) are shown.

Acyl-lipids	Acyl-lipid composition	^{14}C incorporation
LP	1.5 ± 0.3	3.9 ± 0.6
PI	2.7 ± 0.3	1.6 ± 0.2
PC	30.3 ± 4.8	41.2 ± 6.4
PE	20.2 ± 3.7	16.9 ± 3.1
PA	5.0 ± 0.6	10.1 ± 2.6
NL	26.8 ± 6.0	18.9 ± 2.4
MGDG	8.6 ± 1.4	7.4 ± 1.1
DGDG	4.9 ± 0.7	n.d.

n.d. = not detected; LP = Lysophospholipids; PI = Phosphatidylinositol; PC = Phosphatidylcholine; PE = Phosphatidylethanolamine; PA = Phosphatidic-acid; NL = Neutral lipids; MGDG = Monogalactosyldiglycerides; DGDG = Digalactosyldiglycerides.

From the present results and derivative of a previous work (5), it is possible to suggest a similar acylic composition of phospholipids and neutral lipids in microsomes and oil bodies of olive fruit. At the same time, even though in microsomes the galactolipids also appear to be important, the neutral lipids and phospholipids are the major components in both fractions. Apart from the ^{14}C incorporation into oil body and microsome lipids, it might also be possible to indicate that these cellular fractions are two different sites in relation to the lipid synthesis: the oil bodies being for neutral lipids and the microsomes for polyunsaturated fatty-acids and phospholipid biosynthesis. In this sense, other authors (14, 22) suggested the hypothesis that the oil bodies derive from expansions of endoplasmic reticulum membranes, with the triacylglylcerol inserted into hydrofobic layer of the membrane of the endoplasmic reticulum.

In conclusion, it may be possible to suggest that the enzymes implicated in lipid synthesis of the olive fruit oil body, could be contained in a membranous structure associated with this cellular fraction, whose origin might be the endoplasmic reticulum as an active subcellular site of polyunsaturated and phospholipid synthesis. Obviously, much more research with *in vitro* systems is necessary to elucidate the origin and function of oil bodies.

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Resumen

Se estudia la composición y biosíntesis de lípidos en cuerpos grasos y en microsomas de aceituna en las primeras etapas del desarrollo de este fruto. El malonil-CoA-2-C^{14} 4 μm en el medio de incubación de los cuerpos grasos induce una saturación en la velocidad de biosíntesis de ácidos grasos, siendo saturados y monoinsaturados los sintetizados a nivel de fosfolípidos y lípidos neutros. Los lípidos neutros, galactolípidos y, sobre todo, los fosfolípidos son los principales acil-lípidos de microsomas y los ácidos palmítico y oleico sus componentes acílicos mayoritarios. Tras incubar los frutos con acetato-1-C^{14}, los fosfolípidos y los lípidos neutros de microsomas muestran una mayor capacidad de síntesis que los galactolípidos. Aunque en microsomas se sintetizan principalmente ácidos grasos saturados y monoinsaturados, también se observa una síntesis estimable de ácidos poliinsaturados. Estos resultados, en conjunción con estudios morfológicos previamente realizados en este fruto, sugieren la posibilidad de que los cuerpos grasos de la aceituna puedan contener los sintemas enzimáticos necesarios para llevar a cabo la síntesis de los lípidos de reserva.

Palabras clave: *Olea european*, Cuerpos grasos, Microsomas, Biosíntesis de lípidos.

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