# IN VITRO COOPERATION BETWEEN PLASTIDS AND MICROSOMES IN THE BIOSYNTHESIS OF LEAF LIPIDS

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## 1. Introduction

It has been suggested recently that the chloroplast is the major site for de novo synthesis of saturated and monounsaturated fatty acids in plants [1–6]. According to [7,8] plastids are indeed the only sites for the de novo synthesis of acyl groups requiring an obligatory acyl carrier protein. It is well shown by [9–13] that CDP choline and CDP ethanolamine transferases, acyltransferases and desaturases are firmly bound to the endoplasmic reticulum. At the same time, galactosylation and assembly of monogalactosyldiacylglycerol (MGDG) is tightly linked to the chloroplast envelope [14].

Taken together these results suggest that in the plant cells a cooperation between plastids and endoplasmic reticulum membranes is required for the final total synthesis of the membrane lipids.

Here we argue for such a cooperation in an in vitro reconstituted system: intact chloroplasts plus microsomal fractions from spinach leaves.

## 2. Materials and methods

*Spinacia oleracea* (cv. géant d'hiver) plants were grown in short day conditions (9 h white light) for 3 months at 18°C. Plastids were isolated according to [15]. Fresh leaves (60 g) were ground in a Waring blendor with 150 ml sorbitol 0.33 M, EDTA 2 mM, $MgCl_2$ 1 mM, $MnCl_2$ 1 mM, NaCl 20 mM, cysteine HCl 3 mM, in 25 mM MES buffer (pH 6.1). The homogenate filtered through 2 sheets of cheesecloth (miracloth, Touzart et Matignon) was centrifuged at 1000 × *g* for 60 s. The chloroplast pellet was resuspended in the same medium and centrifuged at 200 × *g* for 60 s without braking, then, the supernatant was centrifuged at 2000 × *g* for 60 s and the final purified pellet was resuspended in 1 ml of the same medium containing 25 mM Hepes (pH 6.7) instead of MES. The rate of $CO_2$ fixation determined according to [15] was found to be about 110 μmol $CO_2$ fixed . mg $chl^{-1}$ . $h^{-1}$ indicating that most of the chloroplasts were intact. Broken plastids were obtained by a 1 min sonication at 0°C under $N_2$ flow, or by diluting 4 times the suspension in Hepes buffer without any sorbitol.

The microsomal fraction was the pellet resulting from centrifugation between 25 000 × *g* for 15 min and 100 000 × *g* for 60 min. It contained little chlorophyll and consisted of essentially smooth and rough vesicles of endoplasmic reticulum as seen by electron microscopy. According to [6], chloroplasts (66 μg chl.) were incubated with 2 μCi [1-$^{14}$C]acetate (54.8 μCi/μmol) in the grinding medium containing 25 mM Hepes (pH 7.6) instead of MES. The following cofactors were added: 1 mM ATP, 1 mM CoA and 10 mM sodium bicarbonate. In some experiments, microsomes (480 μg protein), 1 mM UDP galactose or 1 mM G3P were also added in 0.25 ml final vol. Incubations were done in an illuminated

*Abbreviations:* ATP, adenosinetriphosphate; chl, chlorophyll; CoA, coenzyme A; G3P, DL α-glycerophosphate; CDP, cytidine-diphosphate; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; FFA, free fatty acids; 1-2 diG, 1-2 diacylglycerol; BSA, bovine serum albumin; EDTA, ethylenediamine tetraacetate tetrasodium salt; MES, 2(*n*-morpholino)-ethanesulfonic acid; Hepes, *N*-2-hydroxyethylpiperanine-*N'*-2-ethane sulfonic acid; UDP gal, uridine diphosphogalactose

Warburg apparatus shaken at 100 strokes/min.

Incubations were stopped by extraction of lipids [16]. Thin layer chromatography of lipids was done according to [17,18] and the radioactivity was detected by autoradiography. The lipids were scraped off either for scintillation counting or methylation [19]. Gal–liquid radiochromatography of fatty acid methyl esters was done as in [12].

## 3. Results

### 3.1. *Stimulation of acetate incorporation by chloroplast plus microsome mixtures*

The addition of microsomes to intact spinach chloroplasts enhanced markedly the incorporation of [$^{14}$C]acetate into lipids (table 1). As shown by the differences between acetate incorporation into total lipids and total fatty acid methyl esters, this incorporation took place into the polar moiety of the lipid molecules rather than into the fatty acids (table 1). Negligible acetate incorporations into lipids were obtained with microsomes alone or with broken plastids, even in the presence of added microsomes (fig.1).

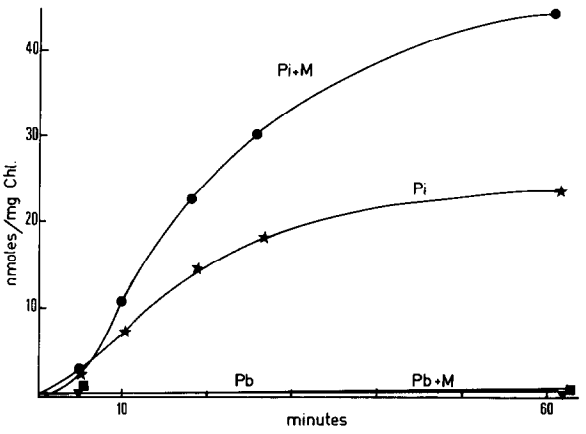


Fig.1. Kinetics of incorporation of [1-$^{14}$C]acetate into total lipids by intact chloroplasts in the absence or in the presence of added microsomes (α-glycerophosphate and UDP-galactose were present in the medium). $P_i$ + M (●), mixtures of intact chloroplasts and microsomes; $P_i$ (★), intact chloroplasts alone; Pb (▼), broken chloroplasts; Pb + M (■), mixtures of broken chloroplasts and microsomes.

### 3.2. *Synthesis of polar lipids in chloroplast plus microsome mixtures*

In the presence of ATP, CoA and $NaHCO_3$ intact chloroplasts incorporated [$^{14}$C]acetate into FFA and 1-2 diG (table 2); very few polar lipids were labelled (<5% of total lipid radioactivity). The addition of microsomes to chloroplasts enhanced the acetate incorporation into FFA and 1-2 diG, but the most striking change observed was a 10-fold stimulation of PC and MGDG synthesis. Traces of labelled PE, PG and triacylglycerols were also found.

When UDP galactose was added to intact chloroplasts, an important acetate incorporation into MGDG occurred, as observed [14,20]. The addition of microsomes leads to the enhancement of the [$^{14}$C]-acetate incorporation not only into PC, MGDG and FFA but also, although to a lesser extent, into the other lipid classes PG, PE and triacylglycerols.

The addition of both G3P and UDP Gal to mixtures of plastids and microsomes resulted in the

Table 1
Stimulation of [1-$^{14}$C]acetate incorporation into total lipids and total fatty acids by addition of microsomes to a preparation of intact spinach chloroplasts

| Additions | Acetate incorporated (nmol/10 mg chl. . h) | | | | | |
|---|---|---|---|---|---|---|
| | Total lipids | | | Total fatty acids | | |
| | M | Cpl | Cpl + M | M | Cpl | Cpl + M |
| None | 0 | 320 | 590 | 0 | 263 | 385 |
| + UDP Gal | 0 | 170 | 630 | 0 | 142 | 489 |
| + UDP Gal + G3P | 0 | 282 | 522 | 0 | 214 | 446 |

*Abbreviations:* M, microsomes; Cpl, chloroplasts

Conditions of incubations are in section 2

Table 2
Stimulation of [1-$^{14}$C]acetate incorporation into various lipidic classes by addition of microsomes to a preparation of intact plastids from spinach leaves

| Additions | Acetate incorporated (nmol/10 mg chl. . h) | | | | | |
|---|---|---|---|---|---|---|
| | None | | + UDP Gal | | + UDP Gal + G3P | |
| | P | P + M | P | P + M | P | P + M |
| PC | 8 | 89 | 3 | 89 | 5 | 105 |
| PG | tr. | 3 | tr. | 3 | tr. | 4 |
| PE | 0 | 8 | 0 | 8 | 0 | 9 |
| MGDG | 3 | 36 | 21 | 79 | 77 | 164 |
| 1-2 diG | 32 | 44 | 6 | 21 | 36 | 58 |
| 1-3 diG | 4 | 10 | tr. | 5 | 3 | 21 |
| FFA | 234 | 368 | 126 | 400 | 122 | 120 |
| Triacyl-glycerols | 0 | 13 | 0 | 9 | 0 | 35 |

*Abbreviations:* P, plastids; M, microsomes

highest rate of synthesis of polar lipids (PC, MGDG) observed in these in vitro systems.

### 3.3. *Nature of the fatty acids synthesized by chloroplast plus microsome mixtures*

The addition of microsomes to intact chloroplasts enhanced the biosynthesis of oleic and palmitic acids (table 3). When G3P was supplied to intact chloroplasts, an increase of palmitic acid synthesis was obtained, independently of the addition of microsomes. The addition of UDP galactose to intact plastids did not change significantly the incorporation of [$^{14}$C]acetate into fatty acids.

When examining the distribution of the labelled fatty acids between the different molecular species, MGDG was found to be richer in [$^{14}$C]palmitate than in [$^{14}$C]oleate, whereas the opposite was true for the neutral lipids and PC (table 4).

## 4. Discussion

Isolated chloroplasts are known to be able to synthesize fatty acids (mainly palmitic and oleic acid) from acetate [4,6]. About 80% of the acetate incorporated is found in FFA and 1-2 diG. Our results agree with these capacities.

When microsomes were added to chloroplasts, an enhancement of lipid synthesis was obtained. But, more interesting is the qualitative modification of the

Table 3
Stimulation of [1-$^{14}$C]acetate incorporation into fatty acids (palmitate C16:0, oleate C18:1, linoleate C18:2 and linolenate C18:3) by addition of microsomes to intact spinach chloroplasts

| Additions | [1-$^{14}$C]Acetate incorporated (nmol/10 mg chl. . h) and | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Palmitate | | Oleate | | Linoleate | | Linolenate | |
| | P | P + M | P | P + M | P | P + M | P | P + M |
| None | 45 | 60 | 190 | 305 | 2 | 3 | tr. | tr. |
| + G3P | 79 | 87 | 117 | 153 | 4 | 4 | tr. | tr. |
| + UDP Gal | 26 | 60 | 90 | 330 | 2 | 7 | tr. | 0.2 |
| + UDP Gal + G3P | 73 | 100 | 100 | 202 | 6 | 12 | tr. | 0.4 |

Table 4
Incorporation of [1-$^{14}$C]acetate into fatty acids (see table 3 for symbols) of phosphatidylcholine, monogalactosyldiacylglycerol and neutral lipids with (P + M) or without (P) addition of microsomes to intact spinach chloroplasts

| | | 1-$^{14}$C]Acetate incorporated (nmol/10 mg chl. . h) and % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PC | | | MGDG | | | Neutral lipids | | |
| | | 16:0 | 18:1 | 18:2 | 16:0 | 18:1 | 18:2 | 16:0 | 18:1 | 18:2 |
| + UDP Gal | P | – | – | – | 6 (71%) | 2 (24%) | 0.4 (5%) | 10 (12%) | 72 (86%) | 2 (2%) |
| | P + M | 3 (9%) | 25 (79%) | 3 (9%) | 4 (63%) | 2 (32%) | 0.3 (5%) | 29 (12%) | 204 (86%) | 5 (2%) |
| + UDP Gal + G3P | P | – | – | – | 23 (64%) | 11 (30%) | 2 (6%) | 29 (28%) | 73 (70%) | 3 (2%) |
| | P + M | 3 (10%) | 22 (76%) | 4 (14%) | 13 (46%) | 12 (43%) | 3 (10%) | 28 (23%) | 89 (73%) | 4 (3%) |

lipid metabolism in the presence of microsomes. Mixtures of microsomes and intact chloroplasts are able to synthesize actively phosphatidylcholine and, to a lesser extent, PG, PE and triacylglycerides. The mixture is also able to synthesize MGDG even in the absence of added UDP galactose.

Thus, the hypothesis of a cooperation between intact chloroplasts and microsomes to obtain all the lipid of the cell is well supported by these results.

The exact mechanism of this cooperation remains to be elucidated. How are the fatty acids synthesized by the plastid exported: as free fatty acids, or inserted in diacylglycerols, or any other complex lipid molecule, or as acyl-CoA or acyl-ACP thioesters? Are lipid exchange proteins implicated in the process? These hypotheses are now under study in our laboratory.

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