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[^{14}C]Acetate incorporation into glycerolipids from cauliflower proplastids and sycamore amyloplasts

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Formation of glycerolipids from [^{14}C]acetate within proplastids from cauliflower buds and amyloplasts from sycamore cells was compared. In cauliflower proplastids, when *sn*-glycerol 3-phosphate was present, most of the radioactivity was recovered as diacylglycerol, which was converted into MGDG after addition of UDP-galactose. The MGDG molecular species synthesized in these conditions was C18:1/C16:0 and was rapidly desaturated into C18:3/C16:0. In sycamore amyloplasts, phosphatidic acid, and not diacylglycerol, was the major compound to be synthesized in the presence of *sn*-glycerol 3-phosphate, therefore, MGDG synthesis from [^{14}C]acetate was not possible in sycamore amyloplasts. We conclude that non-green plastids almost behave like chloroplasts from C16:3 or C18:3 plants as far as lipid biosynthesis is concerned.

Proplastid; Amyloplast; Glycerolipid; Monogalactosyldiacylglycerol; Acetate incorporation; Fatty acid desaturation

1. INTRODUCTION

Plastids (chloroplasts and non-green plastids) are able to incorporate acetate into fatty acids and glycerolipids. However, most of the data concern chloroplasts [1,2] and very little information is available on non-green plastids [2]. Journet and Douce [3] have demonstrated that [^{14}C]acetate could be incorporated into UFA, LPA, PA, DG and MGDG by proplastids isolated from cauliflower buds. However, acetate incorporation into chloroplast glycerolipids has been shown to be different in C16:3 and C18:3 plants [4,5]: chloroplasts from C18:3 plants (such as pea) are unable to incorporate acetate into MGDG, in contrast to chloroplasts from C16:3 plants (such as spinach). In C18:3 plants, MGDG has only the so-called 'eukaryotic' structure of glycerolipids, with C18 fatty acids at both *sn* positions of the glycerol backbone [6]. In C16:3 plants, two types of MGDG are present: in addition to the eukaryotic structure, a 'prokaryotic' structure is present, with C18 fatty acids and C16 fatty acids respectively at the *sn*-1 and the *sn*-2 position of the glycerol backbone [6]. Chloroplasts are probably unable to synthesize the eukaryotic structure of glycerolipids, thus explaining the different biosynthetic properties of chloroplasts from C18:3 and C16:3 plants [1,2,4,5].

The purpose of this article is to determine whether this difference can also be observed in non-green plastids.

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Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; LPA, lysophosphatidic acid; PA, phosphatidic acid; DG, diacylglycerol; UFA, unesterified fatty acids; TLC, thin-layer chromatography

2. MATERIALS AND METHODS

2.1. *Plant material*

Cauliflower (*Brassica oleracea* L.) buds were purchased from local markets. Sycamore (*Acer pseudoplatanus* L.) were grown as a suspension in a liquid medium as in [7].

2.2. *Purification of non-green plastids*

Cauliflower proplastids were prepared using a double Percoll

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purification as described [3]. Sycamore cell plastids were prepared from protoplasts as described [8]. All the non-green plastids used were devoid of any contaminating extraplastidial membranes [3,8]. Non-green plastid integrity was monitored using the assay of the latency of gluconate 6-phosphate dehydrogenase as described in [3]. All preparations containing less than 90% intact plastids were discarded.

2.3. *Incorporation of [^{14}C]acetate into non-green plastids*

Intact and purified non-green plastids (corresponding to 1 mg protein) were incubated at 25°C in 1 ml (final volume) of the following medium: 0.33 M (cauliflower proplastids) or 0.5 M (sycamore amyloplasts) sucrose; 25 mM tricine-NaOH (pH 7.9); 0.13 mM Triton X-100; 10 mM $NaHCO_3$; 2 mM $MgCl_2$; 1 mM dithiothreitol; 0.3 mM Na_2HPO_4; 0.5 mM coenzyme A; 4 mM ATP; 0.5 mM NADP; 10 mM glucose 6-phosphate; 2 U glucose 6-phosphate dehydrogenase (from Boehringer) and 0.5 mM [^{14}C]acetate (1 mCi/mmol). To this basic incubation mixture, 0.5 mM UDP-galactose and/or 1 mM *sn*-glycerol 3-phosphate were added in some experiments. After 15 or 30 min incubation, the reaction was stopped by addition of 10 ml of a chloroform/methanol (1:1, v/v) mixture and the lipids extracted as in [9].

2.4. *Lipid analyses*

After extraction, plastid lipids were separated by two-dimensional TLC as described previously [10], using chloroform/methanol/water (65:25:4, v/v) in the first development and chloroform/acetone/methanol/acetic acid/water (100:40:20:20:10, v/v) in the second development. The radioactive lipids (UFA, DG, PA, LPA, MGDG, etc.) were visualized by autoradiography (X-Omat AR5, Kodak), and scraped off the plates for quantitative determination. Their fatty acid content was analyzed by gas chromatography (IGC 121 DFL, DELSI Instruments) as described [11]. MGDG purified by TLC from cauliflower or sycamore plastids was solubilized in methanol and then analyzed by HPLC.

MGDG molecular species were separated by HPLC and radio-HPLC, essentially as described by Kesselmeier and Heinz [12], using a Varian liquid chromatograph (Model 5000), an UV detector (UV 50, Varian), and a radioactivity monitor (LB 506 D, Berthold). The lipids were separated on an inverse phase column (Spherisorb C6 or C8, 5 μm, Interchim) using a linear acetonitrile gradient (50 to 100% in water) for 30 min (1 ml/min). Elution profiles of intact lipids were recorded at 200 nm. Quantitation was done using the table of relative response factors at 200 and 250 nm for different MGDG molecular species from Kesselmeier and Heinz [12].

2.5. *Other assays*

Proteins were determined according to [13] using bovine serum albumin as a standard. The radioactivity was determined using a scintillation counter (Intertechnique) after addition of 10 ml of ACS medium (Amersham) to the samples to be analyzed.

3. RESULTS

3.1. *Analyses of MGDG molecular species*

Using HPLC, we have first analyzed MGDG molecular species after purification of this lipid by TLC from total lipid extracts obtained from non-green plastids. In cauliflower proplastids, MGDG was formed of two major molecular species containing fatty acids in the following combination: C18:3/C18:3 (49%) and C18:3/C16:3 (48%), minor compounds (1 to 2%) were identified as C18:2/C16:3 and C18:2/C18:2 (fig.1). Using *Rhizopus arrhizus* lipase, we have previously demonstrated that in cauliflower bud plastids, C16:3 fatty acid was exclusively localized at the *sn*-2 position of the glycerol backbone [14]. In contrast, MGDG from sycamore amyloplasts was devoid of molecular species containing C16:3 fatty acid (fig.1). Only molecular species having two C18 fatty acids were demonstrated to be present: 3 distinct peaks of almost similar quantitative importance were obtained by HPLC, one containing C18:3/C18:3 MGDG, one with C18:2/C18:3 MGDG and another one containing C18:1/C18:3 and/or C18:2/C18:2 (which cannot be separated from one another by HPLC). However, since in sycamore amyloplasts MGDG contains only limited amounts (about 7%) of oleic acid [14,15], it is likely that the last peak contained mostly MGDG with a C18:2/C18:2 combination.

These observations demonstrate that cauliflower proplastids contain MGDG having both the eukaryotic structure (C18/C18) and the prokaryotic structure (C18/C16) whereas sycamore amyloplasts have MGDG with a eukaryotic structure only. In chloroplasts, such a difference in the structure of MGDG reflects the biosynthetic pathway for glycerolipid. We thus followed [^{14}C]acetate incorporation into glycerolipids from these non-green plastids.

3.2. *Incorporation of [^{14}C]acetate into plastid lipids*

Table 1 demonstrates that both proplastids from cauliflower buds and amyloplasts from sycamore cells incorporate [^{14}C]acetate into lipids at low (compared to chloroplasts) but significant levels: average rates of 5–10 and 10–50 nmoles acetate·mg protein^{-1}·h^{-1} were obtained respectively for sycamore amyloplasts and cauliflower proplastids, compared to values of 50–150 routinely obtained with spinach chloroplasts. In the absence of *sn*-glycerol 3-phosphate in the incubation mixture, UFA were the major (about

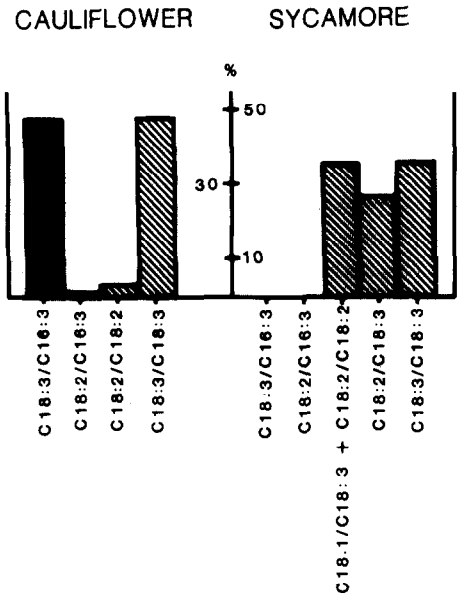

Fig.1. MGDG molecular species in cauliflower and sycamore non-green plastids. MGDG was purified from cauliflower bud proplastids and sycamore cell amyloplasts by two-dimensional TLC. Then, the different molecular species present in the sample were separated by HPLC as described in section 2.

70% of the total radioactivity) compounds to be synthesized in both types of plastids. Analyses of these fatty acids demonstrate that the radioactivity was recovered into 16:0 (palmitic acid) and 18:1 (oleic acid) fatty acids (not shown).

After addition of *sn*-glycerol 3-phosphate, only 25–30% of the total label was recovered as UFA and there was a marked shift of radioactivity incorporated into glycerolipids (table 1). Qualitatively, the glycerolipids formed were identical in cauliflower and sycamore plastids, but they were synthesized in different proportions: the major glycerolipid to be synthesized (about two-thirds of the total radioactivity) was DG in cauliflower proplastids and PA in sycamore amyloplasts. In contrast, only 4–6% of the total radioactivity was present in either DG or PA, respectively in sycamore and in cauliflower (table 1). Analyses of the radioactive fatty acids incorporated into PA and DG (when formed) demonstrate that only 16:0 and 18:1 fatty acids were present. This is in agreement with data obtained with chloroplasts [1].

These results provide further evidence for our observations, using purified envelope membranes from sycamore amyloplasts and cauliflower proplastids [14], that (i) the Kornberg-Pricer pathway responsible for PA synthesis is very efficient in both types of non-green plastids and (ii) amyloplasts from sycamore, in contrast to cauliflower proplastids, have a very low phosphatidate phosphatase activity.

Since a very low level of DG was formed in sycamore amyloplasts after 30 min incubation in the presence of [^{14}C]acetate and *sn*-glycerol 3-phosphate, it is not surprising that MGDG was

Table 1

Fatty acids and glycerolipid biosynthesis from [^{14}C]acetate by cauliflower proplastids and sycamore amyloplasts

Plastid	Reaction mixture	Radioactivity (%) incorporated into						
		LPA	PA	DG	MGDG	DGDG	UFA	Total (nmol acetate/h per mg protein)
Cauliflower proplastid	basic medium	0.7	2.9	28	–	–	67.5	15
	+ glycerol 3-P 1 mM	8	6	66	1	–	18	16
	+ glycerol 3-P 1 mM + UDP-gal 0.5 mM	1.1	9.9	10.5	51	tr	21.9	15.5
Sycamore amyloplast	basic medium	0.5	25	–	–	–	73	4.5
	+ glycerol 3-P 1 mM	5	65	4	–	–	25	5
	+ glycerol 3-P 1 mM + UDP-gal 0.5 mM	2	65	3	1	–	28	5

Plastids from cauliflower buds and sycamore cells were incubated as described in section 2. To the basic incubation mixture, UDP-gal (0.5 mM) and/or *sn*-glycerol 3-phosphate (1 mM) were added. The different lipids were extracted, separated and the radioactivity determined as described in section 2. (–) Not detected; tr, traces (<0.5%)

synthesized only to a very low extent when UDP-gal was added to the incubation mixture (table 1). In contrast, more than 50% of the total radioactivity was recovered in MGDG when UDP-gal was added to cauliflower proplastids (table 1).

3.3. *Desaturation of MGDG fatty acids in cauliflower proplastids*

Desaturation of newly synthesized fatty acids has been observed in isolated intact chloroplasts incubated in the presence of [^{14}C]acetate only at the level of glycerolipids [1]. Since MGDG was synthesized very actively in cauliflower proplastids, we analyzed whether desaturation of fatty acids was also possible in these proplastids.

We have demonstrated, using purified envelope membranes from sycamore amyloplasts and cauliflower proplastids [14,15] that biosynthesis of glycerolipids (PA, DG) through the plastid Kornberg-Pricer pathway led to the formation of the so-called prokaryotic structure, containing 18:1 at the *sn*-1 position and 16:0 at the *sn*-2 position. Indeed, separation by radio-HPLC of newly synthesized MGDG from cauliflower proplastids (fig.2) demonstrates that only one molecular species, containing C18:1/C16:0 fatty acids, was formed after 15 min incubation. Then, after 30 min incubation, a second radioactive peak was detected (fig.2). Careful analyses demonstrate that this molecular species corresponds to MGDG containing a C18:3/C16:0 combination. Therefore, desaturation of C18:1 to C18:2 and C18:3 was possible on MGDG newly synthesized from [^{14}C]acetate in cauliflower proplastids. Surprisingly, desaturation of 16:0 (i.e. at *sn*-2 position) was not detected under our experimental conditions. However, experiments using isolated intact chloroplasts, have shown that desaturation of fatty acids is usually more efficient at the *sn*-1 position (esterified by C18:1) than at the *sn*-2 position of the glycerol backbone.

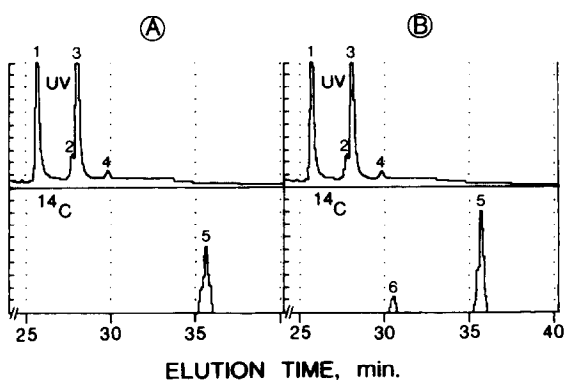

Fig.2. Radio-HPLC analyses of MGDG molecular species synthesized in cauliflower proplastids from [^{14}C]acetate, after 15 min (A) and 30 min (B) incubation. The experimental conditions are described in section 2. The upper traces represent the UV (200 nm) detection (in arbitrary units) of the molecular species. The lower traces represent the detection of radioactive molecular species. The different molecular species separated are: 1, C18:3/C16:3; 2, C18:2/C16:3; 3, C18:3/C18:3; 4, C18:2/C18:2; 5, C18:1/C16:0; 6, C18:3/C16:0.

4. CONCLUSION

The results presented above provide evidence that lipid synthesis in non-green plastids and in chloroplasts occurs following similar pathways. We have demonstrated that proplastids from cauliflower buds and amyloplasts from sycamore cells can incorporate [^{14}C]acetate into UFA (C16:0 and C18:1) to significant levels. These data are in agreement with several observations [3,16–18] which have demonstrated the capacity of non-green plastids to provide fatty acids to the plant cell. Then, after addition of *sn*-glycerol 3-phosphate, a complete shift in the distribution of the radioactivity occurred: more than 70% of the newly synthesized fatty acids were channeled towards glycerolipids (LPA, PA). This is due to the presence, in plastid envelope membranes, of the enzymes from the Kornberg-Pricer pathway [14,15]. However, differences in the proportions of glycerolipids synthesized by non-green plastids (proplastids or amyloplasts) were observed, which could be related, like in chloroplasts, to the plant type (C16:3 or C18:3 plants). In 18:3 plants, MGDG synthesis from [^{14}C]acetate is almost negligible, this is true in sycamore amyloplasts as well as in pea chloroplasts, although envelope membranes from these plastids are able to convert DG into MGDG at high rates when UDP-gal is present [14,15]. The lack of MGDG synthesis in sycamore amyloplasts was due to the low activity of phosphatidate phosphatase in envelope membranes [14], as shown in chloroplasts from C18:3 plants [4,5,19]. In contrast, in 16:3 plants, MGDG synthesis from [^{14}C]acetate, which occurs at the expense of DG, was very powerful, in agreement with our observations that envelope membranes

from cauliflower proplastids [14,15] and spinach chloroplasts [20] are able to synthesize MGDG using *sn*-[^{14}C]glycerol 3-phosphate. In both C18:3 and C16:3 plants, the formation of the eukaryotic structure of glycerolipids could not be demonstrated in vitro using isolated intact plastids. Finally, we have demonstrated that desaturation of fatty acids occurs on the glycerol backbone of MGDG in non-green plastids, in good agreement with the results obtained with isolated intact chloroplasts [1,2,4,5].

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