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Polar Lipid Composition of a Plastid Ribosome-Deficient Barley Mutant[1]

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ALBERT-JEAN DORNE, JEAN-PIERRE CARDE[2], JACQUES JOYARD, THOMAS BÖRNER[3], AND ROLAND DOUCE
Physiologie Cellulaire Végétale, Departement de Recherche Fondamentale/Biologie Végétale, Centre d'Etudes Nucléaaire, and Université Scientifique et Médicale de Grenoble, 85 X, F 38041 Grenoble, Cedex, France

ABSTRACT

Green and white leaves of the barley mutant line 'albostrians' were compared for their polar lipid content and fatty acid composition. The mutant plastids of the white leaves have a double-layered envelope, but in contrast with the normal chloroplasts, lack 70 S ribosomes and thylakoids. In the green leaves, the amount of monogalactosyldiacylglycerol (MGDG) consistently exceeds the amount of digalactosyldiacylglycerol (DGDG) and the amount of galactolipids exceeds the amount of phospholipids. In contrast, in white leaves the amount of DGDG exceeds the amount of MGDG and the amount of phospholipids exceeds the amount of galactolipids. In white leaves, the galactolipid composition reflects the plastid envelope composition which is rich in DGDG, whereas in green leaves the galactolipid composition reflects the thylakoid composition which is rich in MGDG. These results demonstrate the likelihood that all the enzymes involved in galactolipid, sulfolipid and fatty acid synthesis are coded by the nuclear genome.

In plant cells, the occurrence of galactolipids, MGDG[4] and DGDG, is restricted to plastid membranes (6). Both types of chloroplast membranes, envelope and thylakoids, contain galactolipids, but the proportion in which they are present is characteristic for each membrane system: in envelope membranes, the major galactolipid present is DGDG while in thylakoids, it is MGDG (5, 6). Furthermore, it has been demonstrated that the chloroplast envelope is the site of galactolipid synthesis (6). The same is true for nonphotosynthesizing plastids such as chromoplasts or amyloplasts (6). All the enzymes involved in galactolipid synthesis operate in close relation with the fatty acid synthetase complex which is localized in the stroma (5, 6). The question of whether the galactolipid-synthesizing enzymes are themselves coded for by nuclear or chloroplast DNA is an intriguing one, especially in the light of their unique localization in plastid membranes. This question prompted us to analyze the polar lipids of the mutant line "albostrians" of *Hordeum vulgare* L. missing plastid ribosomes and therefore unable to synthesize proteins within the organelle (3). The seedlings obtained from this mutant are entirely green, entirely white, and green-white striped. The apparently white plastids of this line are gene-induced plastome mutants, *i.e.* the mutation of the plastid DNA is induced by a nuclear gene, but is subsequently inherited independently of the nuclear genome (9).

The white leaves contain only mutant plastids which have a double envelope membrane but devoid of ribosomes, of a normal internal membrane system, and of most of the enzymes of the photosynthetic carbon pathway such as RuBPcase (3, 11). In contrast, the green leaves contain normal chloroplasts. In this study, we compare the polar lipid composition of white leaves containing the aberrant plastids with that of green leaves containing normal plastids.

MATERIALS AND METHODS

Plant Material. Leaf material was obtained from the mutant line "albostrians" (M 4205) of *H. vulgare*. Plants (wild-type and mutant) were grown at 22°C in a greenhouse under daylight conditions. Entirely white leaves (from the mutant) and entirely green leaves (from the wild-type) were harvested 6 d after germination.

Lipid Analysis. Leaf aliquots (3–10 g) were fixed by boiling for 5 min in order to destroy phospholipases and lipolytic acyl hydrolases. Leaf lipids were then extracted according to Folch *et al.* (7).

Suitable aliquots were chromatographed in two dimensions on silica gel 60 precoated thin-layer chromatography plates (Merck). A solvent system of chloroform:methanol:H_2O (65:25:4, v/v/v) was used in the first development and chloroform: acetone:methanol:acetic acid:H_2O (100:40:20:20:10, v/v/v/v/v) in the second development. Spots were located under UV light (360 nm) after spraying the plates with anilinonaphthalene sulfonate (0.2% in methanol). Individual polar lipids were identified by their reaction with specific spray reagents and by comparing their R_F values with those of reference standards. Fatty acid methyl esters were made by transesterification of polar lipid fractions at 70°C for 2 h in methanol:sulfuric acid:benzene (100:5:5, v/v/v). Methyl esters were extracted with hexane and chromatographed on an Intersmat gas chromatograph (IGC 131) equipped with a hydrogen flame ionization detector and an Intersmat integrator (ICR-1B). Separations were carried out at 175°C using a column packed with 10% DEGS on chromosorb. Quantitative analysis of fatty acids and their parent lipids were made according to Allen and Good (1).

Electron Microscopy. Small pieces of white and green leaves were prepared for electron microscopy as described by Carde and Launay (4). Observations of the stained thin section were made with EM 300 or EM 301 Philips electron microscopes.

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[2] Present address: Université de Bordeaux I, Laboratoire de Physiologie Cellulaire Végétale, ERA 403, Avenue des Facultés, F 33405 Talence, France.

[3] Present address: Department of Genetics, Section Biosciences, Martin-Luther University, Domplatz 1, DDR-402 HALLE/S, German Democratic Republic.

[4] Abbreviations: MGDG and DGDG, mono- and digalactosyl diacylglycerol; PC, PG, PE, and PI, phosphatidyl-choline, -glycerol, -ethanolamine, and -inositol; DPG, diphosphatidyl glycerol or cardiolipin.

RESULTS AND DISCUSSION

Fine Structure of Plastids from Wild-type and Mutant *Hordeum Vulgare* Leaves. A comparison between the fine structure of a chloroplast from a mature *H. vulgare* leaf and from a leaf of a mutant line 'albostrians' is shown in Figure 1. The normal leaf chloroplast is highly differentiated (Fig. 1a). Its fine structure reveals a level of differentiation equal to that of most of the mature leaf chloroplasts (8). Numerous ribosomes are scattered throughout the stroma (Fig. 1a). These plastid ribosomes are smaller than the cytoplasmic ribosomes (Fig. 1a). In contrast, plastids from the mutant line are characterized by few lamellae, which appear to be formed by the vesiculation of the inner membrane of the plastid envelope (Fig. 1b). Pockets of cytoplasm are found in deep invaginations situated near the periphery of plastids (Fig. 1b). These plastids lack well-organized 70 S ribosomes. This last result, in agreement with previous reports (3, 11),

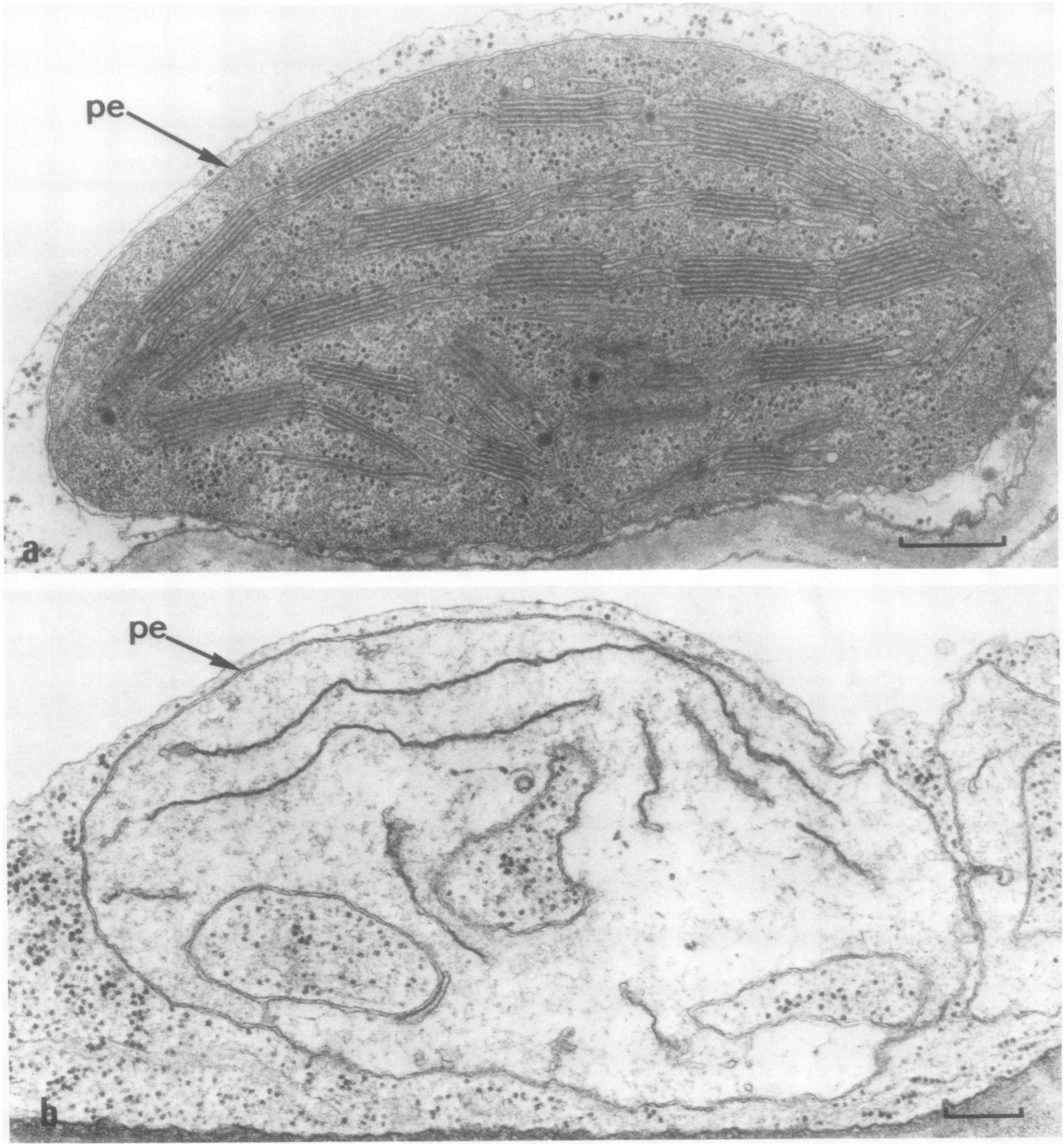

FIG. 1. Fine structure of plastids from wild-type and mutant line 'albostrians' (M 4205) of *H. vulgare*. a, Wild-type: fully differentiated chloroplast with dense stroma, typical 70 S ribosomes and thylakoids, bounded by the two membranes of the envelope (pe) (× 57,000). b, Mutant: plastid with clear stroma devoid of ribosomes is characterized by few lamellae. Note that plastid envelope membranes (pe) are morphologically identical in both plastids. (× 43,000). Scale bar: 0.3 μm.

Table I. *Polar Lipid Composition of 'Albostrians' Mutant Barley Leaves and Wild-Type Barley Leaves*

Polar Lipids	Wild-type		"Albostrians" Mutant	
	μg fatty acids/g fresh wt	%	*μg fatty acids/g fresh wt*	%
MGDG	1,070	35	77	8
DGDG	692	23	124	13
Sulfolipid	111	3.5	10	1
PC	660	22	425	45
PG	291	9	57	6
PE	145	5	210	22
DPG	19	0.5	19	2
PI	57	2	33	3
Total polar lipids	3,045	100	955	100
Total galactolipids	1,762	58	201	21
Total phospholipids	1,172	38.5	744	78

suggests that mutant plastids are unable to synthesize proteins.

Polar Lipid Composition of Wild-type and Mutant Leaves. The polar lipid composition of green and white barley leaves is given in Table I. The mutant has much less polar lipid than the wild-type. The amount of polar lipid in the mutant, when expressed on a weight basis, is only 30% that of wild-type. Furthermore, this reduction is not the same for all the lipid classes: galactolipid, sulfolipid, and PG contents of the mutant are much lower than those measured in the wild-type, whereas PE, PC, and DPG are present in similar amounts in mutant and wild-type. These results are consistent with the finding that (a) galactolipid, sulfolipid, and PG are concentrated in plastid membranes (10), (b) PE and PC are present in large amounts in all the extra-plastidial membranes examined so far (6), and (c) cardiolipin is localized in the mitochondrial inner membrane (2). Therefore, it is likely that the reduction in the content of galactolipid, sulfolipid, and PG reflects the low amount of internal membranes of the mutant plastids as shown in Figure 1.

There is a qualitative as well as a quantitative difference in the galactolipid content of the mutant relative to the wild-type. In the wild-type the major galactolipid is MGDG, whereas in the mutant it is DGDG. This difference reflects the nature of predominant plastid membranes in the barley leaves. In the wild-type having normal chloroplasts, the ratio of internal membrane (thylakoids) to envelope membranes is high (Fig. 1). In this case, the amount to MGDG consistently exceeds the amount of DGDG, and the amount of galactolipids exceeds that of phospholipids. In contrast, as shown in Figure 1, the membrane system of the mutant plastids is almost entirely restricted to the double-layered envelope. In this case, the ratio of envelope membranes to internal membranes is high, the amount of DGDG exceeds the amount of MGDG, and the amount of phospholipids exceeds the amount of galactolipids. In the mutant, the altered ratio of MGDG to DGDG reflects the predominance of the plastid envelope, which is rich in DGDG in all the plastids analyzed so far (for a review, see Ref. 6). In the photosynthetic wild-type, the galactolipid composition reflects the predominance of thylakoid membranes which are rich in MGDG. In support of these suggestions, comparisons of the polar lipid contents of various non-photosynthetic, photosynthetic and greening tissues have led Douce and Joyard (6) to the same conclusion.

Fatty Acid Composition of Polar Lipids from Wild-type and Mutant Leaves. The fatty acid analysis of the polar lipids from mutant and wild-type barley leaves is given in Table II. The total fatty acids are more saturated in the mutant than in the wild-type. The major differences observed are the decrease in the proportions of linolenic acid and the increase in the proportions of linoleic acid. Linolenic acid is still present in the 'albostrians' mutant, thus demonstrating that desaturation of linoleic acid into linolenic acid occurs, even though the plastids lack 70 S ribosomes. However, when each polar lipid is taken separately, the decrease in the proportions of linolenic acid appears to be due to the decrease in the amount of galactolipids. As a matter of fact, the polar lipids localized outside the plastid, such as cardiolipin, have about the same fatty acid composition in both the mutant and the wild-type (Table II). In contrast, in the mutant barley leaves, phosphatidylglycerol has a very different fatty acid composition compared with the wild-type: trans-Δ3-hexadecenoic acid, present in large proportions in the wild-type phosphatidylglycerol is barely detectable in the mutant where an increase in palmitic acid is observed. Trans-Δ3hexadecenoic acid is a specific fatty acid of plastidial phosphatidylglycerol (10). Thus, the fatty acid composition of the mutant leaf phosphatidylglycerol could reflect the absence of thylakoid in the mutant plastid. However, it is likely that all the phosphatidylglycerol found in the white leaves cannot be attributed only to extraplastidial membranes, inasmuch as envelope membranes usually contain this polar lipid (5). Under these conditions, there may be two reasons for the absence of hexadecenoic fatty acid in the mutant phosphatidylglycerol. First, it is not known whether in barley plastids the envelope phosphatidylglycerol actually contains hexadecenoic acid, although this fatty acid is also present in spinach chloroplast envelope membranes (5). Second, the enzyme involved in the desaturation of palmitic acid into trans-Δ3-hexadecenoic acid (or one of its subunit) may be synthesized on plastid ribosomes.

Finally, as shown in Table II, there is little difference between the mutant and the wild-type in the fatty acid composition of MGDG, but this is not true for DGDG. Again, in the mutant, the fatty acid composition of galactolipids reflects the plastid envelope composition in which DGDG is more saturated than in the thylakoids, whereas in the wild-type, the fatty acid composition of galactolipids reflects the thylakoid composition. In accordance with this, analysis of MGDG and DGDG fatty acids in photosynthetic and nonphotosynthetic tissues have led to the same conclusion (for a review see [6]).

CONCLUSION

These results demonstrate that in the barley 'albostrians' mutant devoid of plastid ribosomes, polar lipids specifically localized within the plastid can be synthesized, although in a reduced amount. We assume that the decrease in the galactolipid content of the white leaves is due to the absence of thylakoids in the mutant plastids and not to a reduction of the level of their synthesis, since the galactolipid composition of the mutant leaves reflects the composition of envelope membranes. This is also probably true for phosphatidylglycerol (although there is no trans-Δ3-hexadecenoic acid in the mutant) and for sulfolipid (although the total amount of this lipid in the mutant leaves is very low).

Our observations demonstrate that the apparently normal double layered envelope of the ribosome-deficient plastids contains the same lipids, and in the same proportions, as envelope membranes from various chloroplasts, etioplasts, or amyloplasts (5, 6). This suggests that there is probably a high degree of uniformity in the composition of the envelopes of various types of plastids in plants.

These results also demonstrate that in the albostrians mutant, the absence of plastid ribosomes does not reduce the fatty acid synthesis capabilities of the cell (with, however, the probable exception of trans-Δ3-hexadecenoic acid of envelope phosphatidylglycerol). This result is very important inasmuch as it has been shown that polar lipid synthesis (phospholipids as well as galactolipids) is strongly dependent on plastids. Thus, Olhrogge *et al.* (13) have demonstrated that in the leaf cells, chloroplasts are the sole site for *de novo* fatty acid synthesis. In addition, Nothelfer *et al.* (12) and Vick and Beevers (14) demonstrated that in nonpho-

Table II. *Fatty Acid Composition (% by weight) of the Polar Lipids from 'Albostrians' Mutant Barley Leaves and Wild-type Barley Leaves*

Polar Lipids	Leaves	Fatty Acids					
		C16:0	C16:1	C18:0	C18:1 + C16:3	C18:2	C18:3
MGDG	Wild-type	1	—[a]	—	0.5	3	95
	Mutant	2.5	—	—	0.5	5	91
DGDG	Wild-type	12	—	0.5	1	6	80
	Mutant	18	—	1	3	13	65
SL	Wild-type	26	—	3	1	5	65
	Mutant	20	—	2	1	20	57
PC	Wild-type	21	—	1	5	39	34
	Mutant	19	—	1	4	41	35
PG	Wild-type	25	22	—	2	7	38
	Mutant	42	1	"	1	20	34.5
PE	Wild-type	20	—	1	1	40	38
	Mutant	16	—	0.5	1	48	34.5
DPG	Wild-type	5	—	1	1	20	65
	Mutant	5	—	0.5	1	24	66
Total	Wild-type	16	3	1.2	1.7	17.5	60.6
	Mutant	17.7	0.1	0.7	1.7	24.6	55.2

[a] —, not recorded.

tosynthesizing cells, proplastids are the sole site of fatty acid synthesis. Under these conditions, inasmuch as the mutant barley leaves are competent in both the synthesis of plastid polar lipids and unsaturated fatty acids, it is likely that the enzymes involved in fatty acids, galactolipids, sulfolipid, and phosphatidyl-glycerol synthesis are synthesized on cytoplasmic ribosomes and should enter the chloroplast to reach their functional sites (*i.e.* the thylakoids and the envelope membranes).

LITERATURE CITED

1. ALLEN CF, P GOOD 1971 Acyl lipids in photosynthetic systems. Methods Enzymol 23: 523–547
2. BLIGNY R, R DOUCE 1980 A precise localization of cardiolipin in plant cells. Biochim Biophys Acta 617: 254–263
3. BÖRNER T, B SCHUMANN, R HAGEMANN 1976 Biochemical studies on a plastid ribosome-deficient mutant of *Hordeum vulgare*. *In* Bücher, W Neupert, W Sebald, S Werner, eds, Genetics and Biogenesis of Chloroplasts and Mitochondria. North-Holland, Amsterdam, pp 41–48
4. CARDE JP, J LAUNAY 1980 Fixation des membranes des cellules végétales par l'acide tannique. Biol Cell 38: 13a
5. DOUCE R, J JOYARD 1979 Structure and function of the plastid envelope. Adv Bot Res 7: 1–116
6. DOUCE R, J JOYARD 1980 Plant galactolipids. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 4. Academic Press, New York, pp 321–362
7. FOLCH J, M LEES, GH SLOANE-STANLEY 1957 A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226: 497–509
8. GUNNING BES, MW STEER 1975 Ultrastructure and the Biology of Plant Cells. Edward Arnold, London
9. HAGEMANN R, F SCHOLZ 1962 Ein Fall geninduzierter Mutationen des Plasmotypus bei Gerste. Züchter 32: 50–59
10. HARWOOD JL 1980 Plant acyl lipids: structure, distribution and analysis. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 4. Academic Press, New York, pp 1–55
11. KNOTH R, R HAGEMANN 1977 Struktur and Funktion der genetischen Information in den Plastiden. XVI. Die Feinstruktur der Plastiden und der elektronmikroskopische Nachweis echter Mischzellen in Blattern der Plastonmutationem auslösenden genmutante albostrians von *Hordeum vulgare* L. Biol Zentralbl 96: 141–150
12. NOTHELFER HG, RH BARCKHAUS, F SPENER 1977 Localisation and characterization of the fatty acid synthesizing system in cells of Glycine max (soybean) suspension cultures. Biochim Biophys Acta 489: 370–380
13. OLHROGGE JB, DA KUHN, PK STUMPF 1979 Subcellular localization of acyl carrier protein in leaf protoplasts of *spinacia oleracea*. Proc Natl Acad Sci USA 76: 1194–1198
14. VICK B, H BEEVERS 1978 Fatty acid synthesis in endosperm of young castor bean seedlings. Plant Physiol 62: 173–178