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Lipid Composition of Envelopes, Prolamellar Bodies and Other Plastid Membranes in Etiolated, Green and Greening Wheat Leaves*

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Summary. Comparative studies of lipid composition were made on prolamellar bodies, envelopes and other plastid membranes separately extracted from etiolated, green or greening (intermittent or continuous light) wheat (*Triticum sativum* L.) leaves. The different membrane fractions were examined by electron microscopy.

The major lipid was digalactosyldiglyceride in the envelopes and prolamellar bodies and monogalactosyldiglyceride in stroma lamellae and grana. Phosphatidylcholine represented 60% of total phospholipids in the envelopes, 30% in prolamellar bodies and 14% in grana. All types of envelopes had the same lipid proportions.

For all lipids the lowest fatty acid unsaturation was always found in the envelope membranes. The relative amount of trans-Δ_3-hexadecenoic acid in the phosphatidylglycerol of envelopes increased from 4% (etioplasts) to an average of 15% (etiochloroplasts and chloroplasts).

Introduction

Some data about the biochemical composition of chloroplast membranes have been reported (Mackender and Leech, 1972, 1974; Allen et al., 1972; Douce et al., 1973; Poincelot, 1973). Changes in plastid envelope polypeptides during greening were described by Cobb and Wellburn (1974). After studying the lipid composition of intact plastids isolated from the first leaf of wheat grown either in darkness or under different light treatments (Bahl et al., 1974, 1975), the aim of this work was to extend the analysis of lipids to envelopes and other plastid membranes: prolamellar bodies (etioplasts), prolamellar bodies and primary thylakoids (etiochloroplasts isolated from leaves grown under intermittent light), stroma lamellae and grana stacks (etiochloroplasts isolated from etiolated leaves submitted to greening for 24 h under continuous light), chloroplast stroma lamellae and grana.

* *Abbreviations*: DGDG = digalactosyldiglyceride; MGDG = monogalactosyldiglyceride; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; SL = sulfolipid

Materials and Methods

Plant Material and Growing Conditions

Wheat (*Triticum sativum*, var. "Florence Aurore") was sown in vermiculite to which nutrient solution was added (Coic, 1961). Germination and growth were carried out at 21°C under different conditions: total darkness (etioplasts E), continuous light (chloroplasts C), darkness followed by intermittent light (1 ms flashes of ca 83 W m^{-2} alternating with 15 min dark periods) for the last 3 days (etiochloroplasts ECi), darkness plus continuous light (44 W m^{-2}) for the last 24 h (etiochloroplasts ECc). Leaves were always harvested 8 days after sowing.

Plastid Isolation

Plastids were extracted from the tip and middle part of the freshly harvested leaves by procedures previously described as technique E for etioplasts and etiochloroplasts, and as technique C for chloroplasts (Bahl et al., 1974). They were intact and free from mitochondrial or bacterial contamination.

Isolation of Prolamellar Bodies, Envelopes and Other Plastid Membrane Fractions

The rupture of intact plastids was done by a gentle osmotic shock and suspensions were loaded on sucrose gradients according to a method described by Douce et al. (1973). The centrifugation (1 h at 52,500 *g*) in a swinging bucket rotor (Beckman model L3-50, SW 27:1 rotor) resulted in a separation of membranes into 2 or 3 bands on the discontinuous sucrose gradient (see Fig. 1 under "Results"). Separated membrane fractions were removed, diluted four times with buffer and spun 1 h at 100,000 *g* (Beckman model L3-50, 42:1 rotor).

Vol (ml)	Sucrose (M)	Fraction N°	Etioplasts (E)	Etiochloroplasts		Chloroplasts (C) R_c=2.3
				(ECi), R_c=4.6	(ECc), R_c=2.2	
10	0.05					
6	0.60					
		1	Envelopes	Envelopes	Envelopes	Envelopes
6	0.93					
		2	Prolamellar bodies	Prolamellar bodies +primary thylakoids	Stroma lamellae	Stroma lamellae
6	1.20					
		3	Few prolamellar bodies	Few prolamellar bodies +primary thylakoids	Grana	Grana
6	1.50					

Fig. 1. Different fractions of membranes obtained on a discontinuous sucrose gradient. R_c=chlorophyll a/chlorophyll b ratio

Electron Microscopy

The material was fixed with 4% glutaraldehyde in 70 mM sodium cacodylate buffer (pH 7.4) for 12 h at 4° C and was post-fixed in 2% buffered osmium tetroxide for 2 h at 4°C. After dehydration in a series of graded ethanols, specimens were embedded in araldite or in Epon 812 (Luft, 1961). Sections were prepared using a LKB ultramicrotome and they were stained either with uranyl-acetate and lead citrate (Reynolds, 1963) or with potassium permanganate and examined under a JEM 7 electron microscope.

Lipid Extraction and Analysis

Lipids were extracted with chloroform/methanol 2/1 v/v according to the method of Folch et al. (1957) then separated by one dimensional TLC on Silica Gel HR (method of Pohl et al., 1970). Galactolipids, sulfolipid and phospholipids were visualized with primuline 1 mg/100 ml (Folch et al., 1957) in acetone/water 80/20 v/v. Fatty acid methyl esters were prepared by transesterification in methanol/benzene/sulfuric acid 20/10/1 v/v at 70° for 90 min. Quantitative and qualitative analysises of fatty acids were carried out by gas chromatography as previously described (Lechevallier et al., 1972). Peak detection and area integration were performed by autolab "System IV" computing integrator for chromatography. Fatty acid determinations were reproducible to within ±1%.

Results

Electron Microscopy

The main membrane fractions found on the discontinuous gradient were examined by electron microscopy. The results are summarized in Figure 1. For all kinds of plastids, the purified envelope membranes are localized on the discontinuous sucrose gradient at the interface of the 0.60 and 0.93 M layers (fraction 1) as a band of pale yellow color. When examined in the electron microscope, the pellet consisted of pure single and double membrane vesicles (Fig. 2A, 2B).

Prolamellar bodies isolated from etioplasts E (Fig. 3A) and from etiochloroplasts ECi (Fig. 3B) were mainly localized at the interface of the 0.93 M and 1.20 M sucrose layers (fraction 2), however some of them might pass through the 1.20 M layer during the course of the centrifugation (fraction 3). These fractions contained prolamellar bodies without lamellar extensions when isolated from etioplasts, and both crystalline and loose dispersing prolamellar bodies with lamellar extensions, when extracted from etiochloroplasts.

Stroma lamellae prepared from etiochloroplasts ECc (Fig. 4A) and from chloroplasts C (Fig. 4B) were localized in fraction 2. They consisted exclusively of large unfused stacks of parallel thylakoids, very poor in plastoglobuli.

Small grana stacks isolated from etiochloroplasts ECc (Fig. 5A) and well-developed grana from chloroplasts C (Fig. 5B) were localized after centrifugation at the interface 1.20–1.50 M (fraction 3). Grana were slightly swollen probably due to osmotic shock. Between the grana stacks, some stroma lamellae were present.

Lipid Analysis

Results reported in Table 1 (lipid composition per cent by weight) and Table 2 (fatty acid proportions of each lipid) give the mean value of 3 experiments (variability: ±3%). For etioplasts E and etiochloroplasts ECi, the lipid and fatty acid relative concentration in fraction 3 did not differ from that in fraction 2. So only the latter one is reported here.

In the prolamellar body (etioplasts E), DGDG was the major lipid and the MGDG/DGDG ratio was 0.8; galactolipids represented 77% of the total lipids and PC, 30% of the phospholipids. Very slight changes occured in lipid proportions when the prolamellar body gave rise to the first lamellae (ECi): only the relative amount of PC and PG decreased slightly. The same result has been obtained after 2 h of greening under continuous light (Bahl and Monéger).

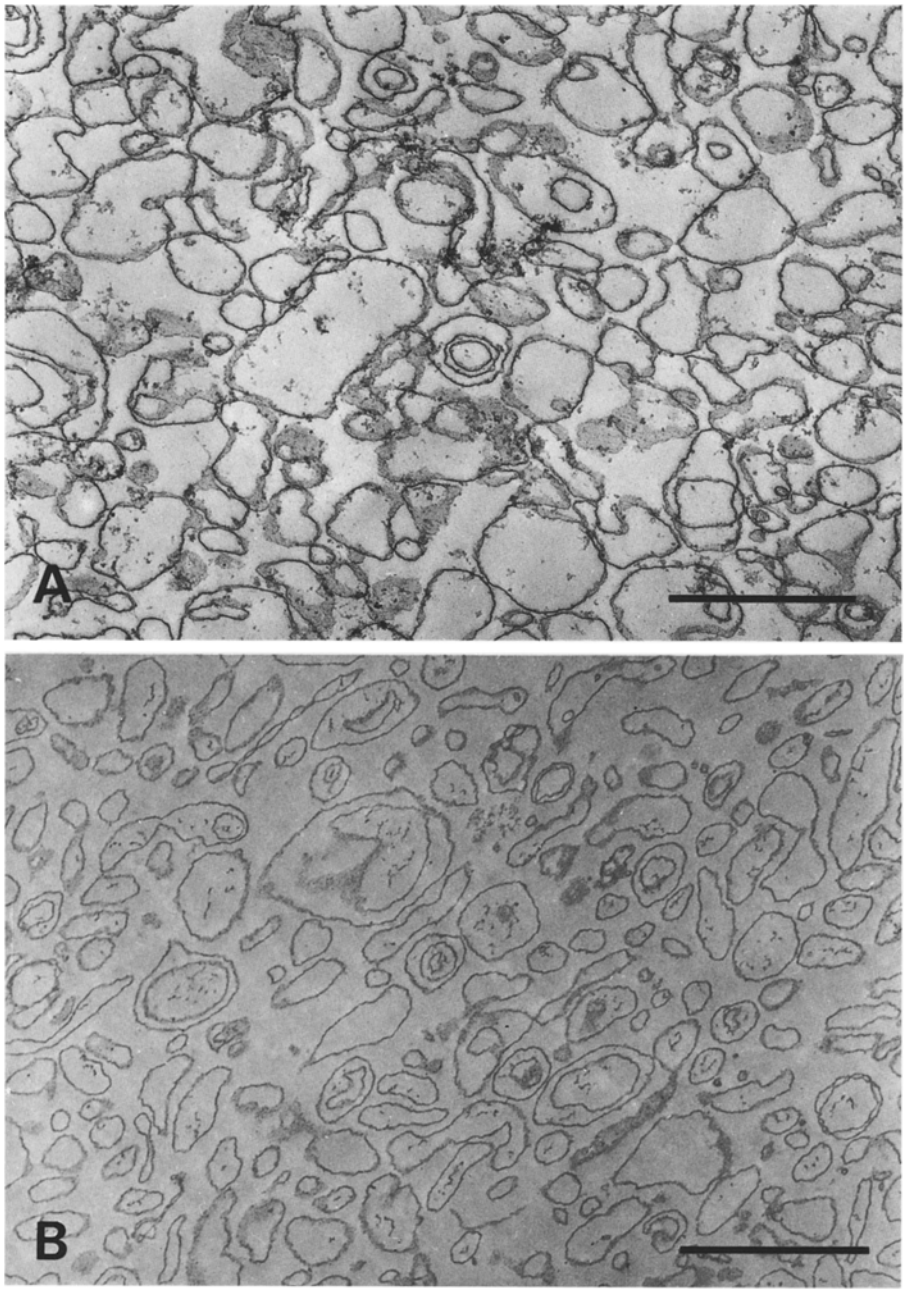

Fig. 2. (A) Plastid envelopes isolated from 8 days old dark grown wheat leaves. The pellet was embedded in araldite, sections were post-stained with $KMnO_4$ (×25,000). (B) Chloroplast envelopes isolated from 8 days old green leaves, embedded in epon, stained with uranyl acetate and lead citrate (×25,000). Marker bars represent 1 μm

After 24 h of continuous illumination, the etiochloroplasts (ECc) possessed a lamellar system already well differentiated. In the stroma lamellae, MGDG and DGDG were present in about equal amounts. In the grana stacks, MGDG was the main galactolipid and the relative concentration of PG, SL and PC was lower than in stroma lamellae.

In chloroplast (C) lamellae, MGDG was the major lipid. This is reflected by the MGDG/DGDG ratio which is 1.1 in stroma lamellae and 1.3 in grana membranes. For both types of membranes, the relative concentration of PC was very low (especially in grana stacks) and the amount of SL was smaller than the amount of PG.

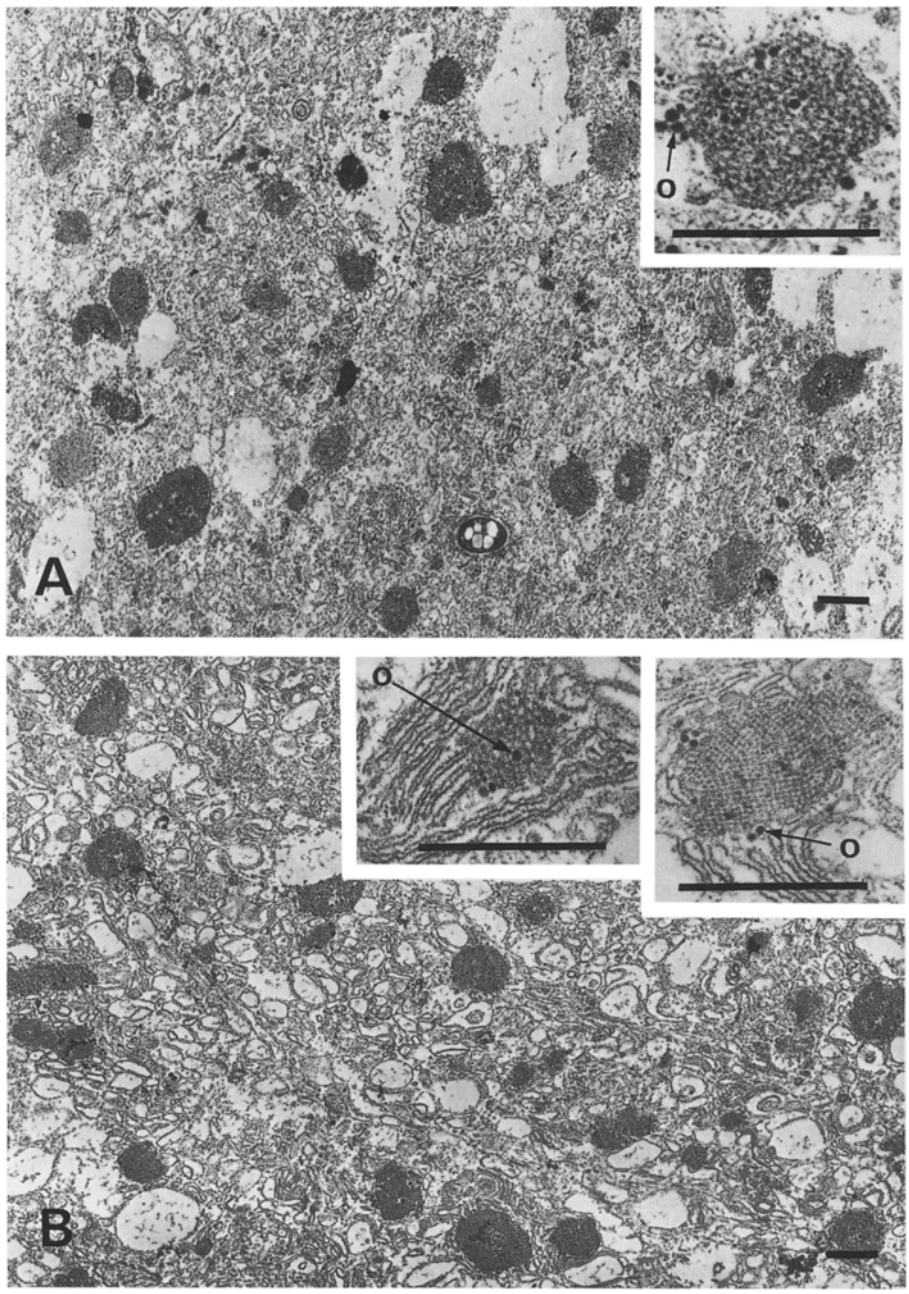

Fig. 3. (A) A representative field (×7,000) and an enlarged section of the prolamellar body fraction (×28,000) isolated from 8 days old dark grown wheat leaves (Araldite–uranyl acetate and lead citrate). (B) Prolamellar bodies and thylakoids from 8 days old etiolated seedlings leaves greened for the last 3 days intermittent light (1 ms flashes alternating with 15 min dark periods) (×7,000): Inset, prolamellar bodies with lateral thylakoid extensions at two different developmental stages (×25,000) (Araldite-$KMnO_4$). *O*: osmiophilic droplets. Marker bars represent 1 μm

The lipid proportions were the same for all types of envelope membranes (Table 1). Galactolipids represented only 65% of the main lipids with a higher relative concentration of DGDG (MGDG/DGDG ratio was 0.5). PC was the major phospholipid (60%).

Galactolipid and sulfolipid fatty acids were qualitatively similar in prolamellar membranes, stroma lamellae and grana. PC had the same fatty acid composition in prolamellar body and in stroma lamellae. However, in grana, its unsaturation increased markedly (linolenic acid became the major fatty acid). In prolamellar membranes, the trans-Δ_3-hexadecenoic

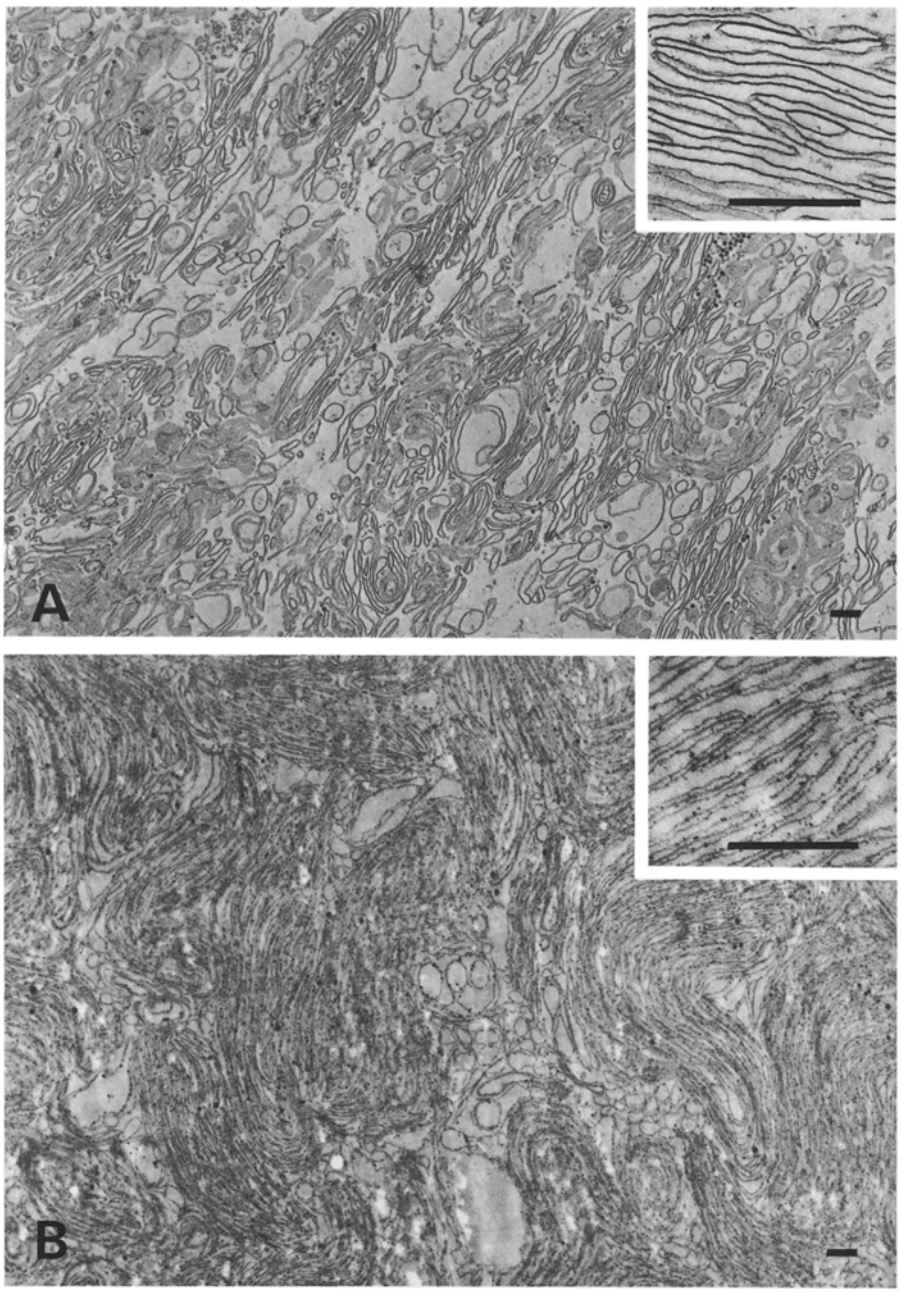

Fig. 4. (A) A low magnification (×7,800) and an enlarged section (×35,000) of the stroma lamellae fraction isolated from 8 days old dark grown wheat leaves illuminated continuously for the last 24 h (Araldite-$KMnO_4$). (B) A low magnification (×7,800) and an enlarged section (×35,000) of the stroma lamellae fraction isolated from chloroplasts of 8 days old green leaves. (Epon – uranyl acetate and lead citrate). Marker bars represent 0.5 μm

acid represented already 11% of total fatty acids in PG and this proportion increased as thylakoids were formed.

The fatty acid composition of the envelope lipids (Table 2), with the exception of PG, was the same in all types of plastids, and compared with other plastid membranes, there was proportionally more palmitic acid and less linolenic acid. In PG of etioplast envelopes, trans-Δ_3-hexadecenoic acid was found in a significant amount (4%). However these envelope membranes contained a higher relative amount of palmitic and linoleic acids and a lower amount of

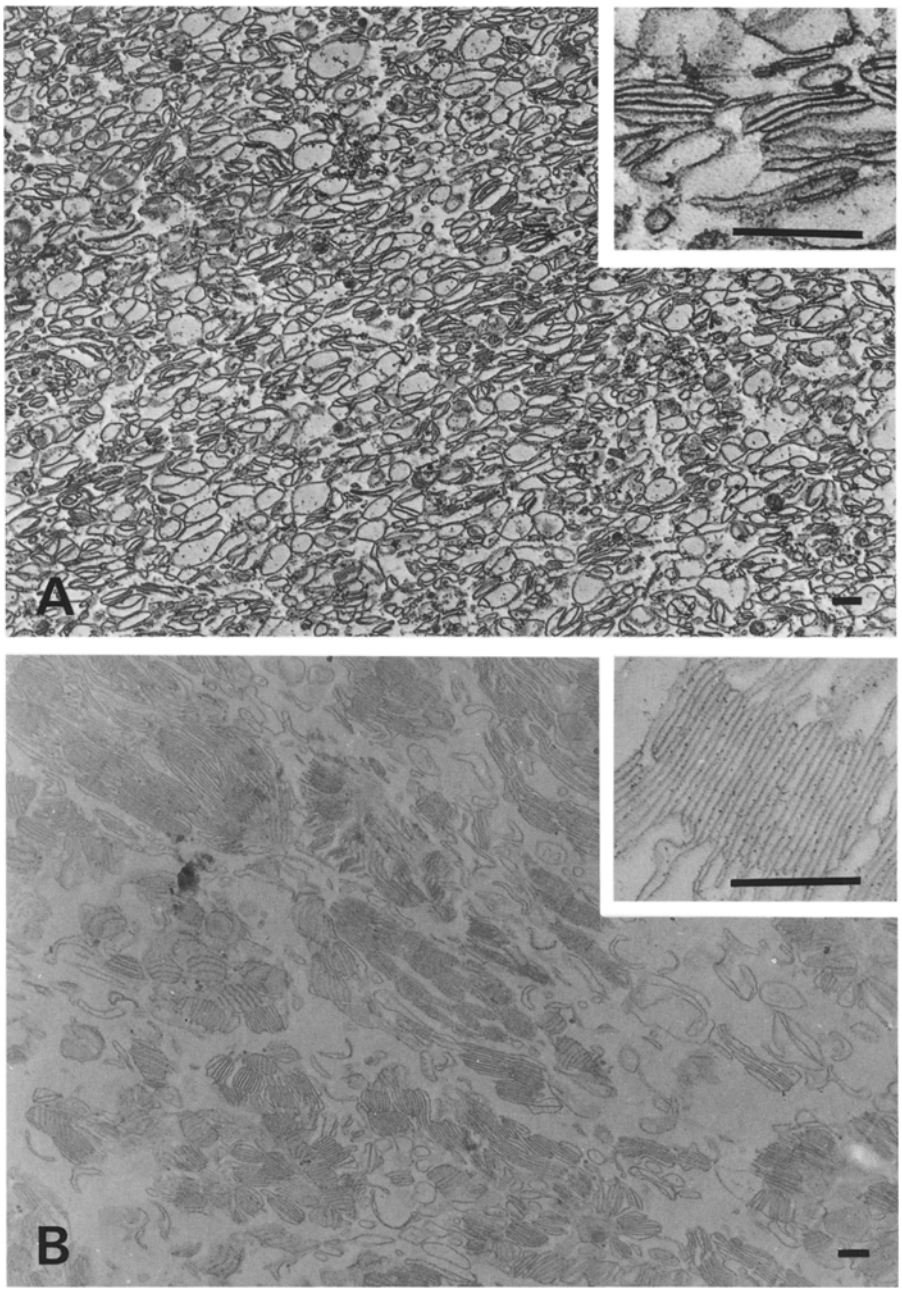

Fig. 5. (A) A low magnification (×7,800) and an enlarged section (×35,000) of the grana fraction isolated from plastids of 8 days old etiolated wheat leaves illuminated continuously for the last 24 h (Araldite-$KMnO_4$). (B) A low magnification (×7,800) and an enlarged section (×35,000) of the grana fraction isolated from chloroplasts of 8 days old green leaves (Araldite–uranyl acetate and lead citrate). Marker bars represent 0.5 μm

hexadecenoic and linolenic acids in PG than etiochloroplast and chloroplast envelopes.

Discussion

In previous papers (Bahl et al., 1974, 1975), we have reported on the lipid composition of plastids isolated from etiolated, green and greening leaves. The aim of the present work was to analyse the distribution of lipids in the different constitutive membranes of those organelles.

The envelope fractions of mature plastids, isolated from wheat leaves grown in darkness or greened under different light treatments, consisted of single or double membrane vesicles, free from other plastid

Table 1. Lipid composition (per cent by weight) of envelopes, prolamellar and lamellar membranes isolated from intact etioplasts, etiochloroplasts and chloroplasts

	MGDG	DGDG	PG	SL	PC
Envelopes					
E	22	44	9.3	10	14
ECi	20.5	45	9.6	11.3	13.5
ECc	21	38.5	11	13.1	16
C	22	43.3	9.8	11.1	13.7
Prolamellar and lamellar membranes					
E fraction 2	36	41	8.6	10	3.7
ECi fraction 2	36	44	7.3	10	2.5
ECc fraction 2	39.5	39	9	10	2.1
fraction 3	43	40	7	8	1.5
C fraction 2	42	37	10	8.5	2.4
fraction 3	47.4	36	8.6	6.6	1.4

C=chloroplasts (fraction 2: stroma lamellae; fraction 3: grana). ECi=etiochloroplasts isolated from leaves greened under intermittent light (fraction 2: prolamellar bodies+thylakoids). ECc=etiochloroplasts isolated from leaves greened under continuous light (fraction 2: stroma lamellae; fraction 3: grana). E=etioplasts (fraction 2: prolamellar bodies).

membranes. Double membrane vesicles might correspond to the inner and outer plastid envelopes, but it is not possible to determine the origin of the single membrane vesicles. The appearance and size of wheat plastid envelopes were similar to those of other chloroplasts isolated from spinach, Vicia faba or Avena sativa. We found a high relative proportion of DGDG, about twice the amount of MGDG in these envelopes. This is in good agreement with results published by Douce et al. (1973) and slightly different from those of Mackender and Leech (1971) and Poincelot (1973). PC, although the predominant phospholipid, was found in a lower relative amount (60% of phospholipid) as compared with spinach (77%) (Douce et al., 1973), or Vicia faba (76%) (Mackender and Leech, 1974). There was no trace of PS, PI or PE. SL was present in significant proportions (10%) in envelope membranes, as reported for spinach plastid envelopes (Douce et al., 1973), but in contrast with another publication on the same material where SL was barely detectable (Poincelot, 1973).

Table 2. Fatty acid composition (per cent by weight) of envelopes, prolamellar and lamellar membranes isolated from intact etioplasts, etiochloroplasts and chloroplasts

		Envelopes						Prolamellar and lamellar membranes					
		C_{16_0}	C_{16_1}	C_{18_0}	C_{18_1}	C_{18_2}	C_{18_3}	C_{16_0}	C_{16_1}	C_{18_0}	C_{18_1}	C_{18_2}	C_{18_3}
MGDG	E	7	–	1	2	3	86	1	–	Tr	Tr	3	95
	ECi	7	–	1	2	3	86	1	–	Tr	Tr	3	95
	ECc	7	–	1	2	3	86	1	–	Tr	Tr	3	95
	C	7	–	1	2	3	86	1§	–	Tr§	Tr§	3§	95§
								1§§	–	Tr§§	Tr§§	3§§	95§§
DGDG	E	14	–	1	2	4	79	7	–	Tr	Tr	3	88
	ECi	14	–	1	2	4	79	7	–	Tr	Tr	3	88
	ECc	14	–	1	2	4	79	7	–	Tr	Tr	3	88
	C	14	–	1	2	4	79	7§	–	Tr§	Tr§	3§	88§
								7§§	–	–	–	3§§	88§§
PG	E	48	4	2	3	10	32	44	11	1	2	3	38
	ECi	40	15	1	2	4	38	26	22	1	2	4	43
	ECc	40	15	1	2	4	38	26	22	1	2	4	43
	C	40	15	1	2	4	38	26§	22§	1§	2§	4§	43§
								26§§	22§§	1§§	2§§	4§§	43§§
SL	E	38	–	Tr	3	4	53	28	–	1	1	4	66
	ECi	38	–	Tr	3	4	53	28	–	1	1	4	66
	ECc	38	–	Tr	3	4	53	28	–	1	1	4	66
	C	38	–	Tr	3	4	53	28§	–	1§	1§	4§	66§
								28§§	–	1§§	1§§	4§§	66§§
PC	E	30	–	2	5	35	27	32	–	2	8	30	27
	ECi	30	–	2	5	35	27	32	–	2	8	30	27
	ECc	30	–	2	5	35	27	32	–	2	8	30	27
	C	30	–	2	5	35	27	32§	–	2§	8§	30§	27§
								27§§	–	2§§	4§§	30§§	37§§

C=chloroplasts (fraction 1: envelopes; fraction 2§: stroma lamellae; fraction 3§§: grana). E=etioplasts (fraction 1: envelopes; fraction 2: prolamellar bodies). ECc=etiochloroplasts greened under continuous light (fraction 1: envelopes; fraction 2: stroma lamellae). ECi=etiochloroplasts greened under intermittent light (fraction 1: envelopes; fraction 2: prolamellar bodies+thylakoids). Tr=traces<1%.

For each lipid, when the variations in fatty acid proportion observed with different light treatments fell within the range of experimental error, only the mean value was given.

The fatty acids of the envelope lipids, PC excepted, were more saturated than in the other membranes. This is in agreement with other published data: the fatty acids of galactolipids in Vicia faba (Mackender and Leech, 1974) and of DGDG, PG, PC in spinach (Douce et al., 1973) chloroplast envelopes were more saturated than in lipids of chloroplast lamellae. Trans-Δ_3-hexadecenoic acid had not been detected in lipids of chloroplast envelopes of Vicia faba (Mackender and Leech, 1974), but these authors have analysed SL, PC and PG together. If traces of this acid were present, it seems likely that it would be detectable only if PG was analysed separately. In spinach chloroplast envelopes, trans-Δ_3-hexadecenoic acid was one of the major fatty acids (35%) of PG (Douce et al., 1973). In wheat chloroplast envelopes it represented only 23%. As we have reported previously (Bahl et al., 1974) light is not necessary to induce the synthesis of trans-Δ_3-hexadecenoic acid, which is already present in envelopes of etioplasts (4%) and in prolamellar membranes (11%).

To our knowledge, no lipid analysis of the prolamellar body has been reported so far. The high percentage of DGDG in prolamellar membranes corresponds to the relative amount of this lipid already found in etioplasts (Bahl et al., 1974). It is also interesting to note that the proportion of PC is much lower than that in the envelope membranes, but higher than that in the lamellae fractions. Under light, the prolamellar body disappeared and thylakoids were differenciated; this was accompanied by an increase in the relative proportion of MGDG with concomitant decrease of DGDG, SL and PC.

An enrichment in MGDG accompanied the grana system formation in wheat plastids while it did not seem to be the case in spinach chloroplasts (Allen et al., 1972) where grana and stroma lamellae contained about the same proportion of MGDG and DGDG. However, a high percentage of MGDG has been found in grana of spinach where MGDG/DGDG=2 (Douce et al., 1973) and of Vicia faba where MGDG/DGDG=2.5 (Mackender and Leech, 1974).

The relative content of phospholipids and especially sulfolipid was lower in grana membranes than in stroma lamellae, which is in agreement with other data (Douce et al., 1973). Only in chloroplast stroma and grana membranes was SL found to be in less amount than PG, confirming our previous results (Bahl et al., 1974). A slight amount of PC was present in grana membranes where this lipid had a special fatty acid composition, less C_{16-0} and more C_{18-3} than PC of other membranes. The same results had been found for spinach chloroplasts (Douce et al., 1973). The high unsaturation of the PC fatty acids in grana might correspond to the reduced function which could be expected there for this lipid (on account of its concentration, much lower in grana than in envelopes, prolamellar bodies and stroma lamellae).

In conclusion, it is interesting to note the similarities between the lipid and fatty acid composition, PG excepted, of the envelopes isolated from etioplasts, etiochloroplasts and chloroplasts, although the metabolite exchanges between plastids and cytoplasm would be expected to differ, depending whether photosynthesis occurs or not. When the leaves are grown in darkness, the prolamellar bodies formed within the plastids have a composition intermediate between the envelopes and chloroplast lamellae. During the course of etioplast development in the light, the relative amount of trans-Δ_3-hexadecenoic acid in PG of envelopes and other membranes rapidly reaches values equal to that in chloroplast. The proportion of MGDG increases in thylakoid membranes, where this component becomes the major lipid. The minute proportion of PC in chloroplast grana and its high percentage in envelope membranes might be explained by its involvement in fatty acid transfer and conversion (Gurr and Brawn, 1970). Work is now in progress to determine lipid changes in plastid membranes when etioplasts are submitted to greening under different light treatments.

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References

Allen, C.F., Good, P., Trosper, T., Park, R.B.: Chlorophyll, Glycerolipid and Protein ratios in Spinach chloroplast grana and stroma lamellae. Biochem. biophys. Res. Commun. **48**, 907–913 (1972)

Bahl, J., Lechevallier, D., Monéger, R.: Etude comparée de l'évolution à l'obscurité et à la lumière des lipides plastidiaux de feuilles étiolées de Blé. Physiol. vég. **12**, 229–249 (1974)

Bahl, J., Phung-Nhu-Hung, S., Lechevallier, D. Monéger, R.: Effets de la lumière intermittente suivie ou non d'éclairement continu sur les lipides plastidiaux de feuilles étiolées de Blé. Comportement particulier du phosphatidylglycérol. Physiol. vég. **13**, 115–124 (1975)

Bahl, J., Monéger, R.: Sur les lipides des corps prolamellaires isolés de feuilles de Blé étiolées et verdissantes. C. R. Acad. Sci. (Paris) **282**, 177–180 (1976)

Cobb, A.H., Wellburn, A.R.: Changes in plastid envelope polypeptides during chloroplast development. Planta (Berl.) **121**, 273–283 (1974)

Coic, Y.: Comparaison de l'influence de la nutrition nitrique et ammoniacale combinée ou non avec une déficience en acide phosphorique sur l'absorption et la métabolisme des anions, cations et plus particulièrement des acides organiques chez le Maïs. Comparaison du Maïs et de la Tomate quant à l'essai de la nature de l'alimentation azotée. Ann. Physiol. vég. **3**, 141–163 (1961)

Douce, R., Holtz, R.B., Benson, A.A.: Isolation and properties of the envelope of Spinach chloroplasts. J. biol. Chem. **248**, 7215–7222 (1973)

Folch, J., Lees, M., Sloane Stanley, G.H.: A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. **226**, 497–509 (1957)

Gurr, M.I., Brawn, P.: The biosynthesis of polyunsaturated fatty acids by photosynthetic tissue. Europ. J. Biochem. **17**, 19–22 (1970)

Lechevallier, D., Bahl, J., Monéger, R.: Etude microanalytique des lipides dans de petits échantillons d'étioplastes et de chloroplastes isolés de feuilles de Blé. C. R. Acad. Sci. (Paris) **272**, 963–966 (1972)

Luft, J.H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961)

Mackender, R.O., Leech, R.M.: Isolation and characterization of plastid envelope membranes. In: Proc. IInd Int. Congr. Photosynthetis Res. Stresa, Vol. 2, pp. 1431–1440, Forti G., Avron M., Melandri A., (ed.), The Hague: Dr. W. Junk NV 1972

Mackender, R.O., Leech, R.: The galactolipid, phospholipid, and fatty acid composition of the chloroplast envelope membranes of *Vicia faba* L. Plant Physiol. **53**, 496–502 (1974)

Pohl, P., Glasl, H., Wagner, H.: Zur analytik pflanzlicher glyko und phospholipoid und ihrer fettsäuren. I. Eine neue dünnschichtchromatographische methode zur trennung pflanzlicher lipoide und quantitativen bestimmung ihrer fettsäurezusammensetzung. J. Chromatog. **49**, 488–49 (1970)

Poincelot, R.: Isolation and lipid composition of Spinach chloroplast envelope membranes. Arch. Biochem. Biophys. **159**, 134–142 (1973)

Reynolds, E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963)

Wright, R.S.: A reagent for the non destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms. J. Chromatog. **59**, 220–221 (1971)

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