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Localization of Galactolipid Biosynthesis in Etioplasts Isolated from Dark-Grown Wheat (*Triticum aestivum* L.)[1]

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ABSTRACT

Etioplasts were isolated from leaves of dark-grown wheat (*Triticum aestivum* L. var Starke II). Galactolipid biosynthesis was assayed in an envelope-rich fraction and in the fraction containing the rest of the etioplast membranes by measuring incorporation of ^{14}C from uridine-diphospho[^{14}C]galactose into monogalactosyl diacylglycerol and digalactosyl diacylglycerol. More than half of the galactolipid biosynthetic capability was found in the fraction of inner etioplast membranes. This fraction was subfractioned into fractions enriched in prolamellar bodies and membrane vesicles (prothylakoids), respectively. All membrane fractions obtained from etioplasts were able to carry out galactolipid biosynthesis, although the activity was very low in prolamellar body-enriched fractions. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed markedly different polypeptide patterns between the different fractions. It is concluded that the capability of galactolipid biosynthesis of etioplasts probably is not restricted to the envelope, but is also present in the inner membranes of this plastid.

Monogalactosyl diacylglycerol (MGDG[2]), the major lipid class of chloroplasts of higher plants (17), is synthesized in the envelope of the chloroplasts (10) by a transfer of a galactose unit from UDP-galactose (synthesized outside the chloroplast [2]) to a diacylglycerol molecule (20). The newly synthesized MGDG molecules are rapidly transported from the envelope to the stroma and grana thylakoids (2, 21), or utilized as substrate for synthesis of DGDG (13, 32, 38). The enzymes synthesizing MGDG and DGDG are generally considered to be located in the envelope of the chloroplast (13), although Williams *et al.* (42) and Gillanders *et al.* (15) do not exclude the possibility that galactosyl transferase activity to a minor extent also may be present in stroma thylakoids and/or grana thylakoids.

The ultrastructural relationship between the envelope membranes and the inner membranes of plastids has been extensively studied (12, 34). During the early stages of plastid development, invaginations occur from the inner membrane of the proplastid envelope (11, 41). Later in chloroplast development, these invaginations become more and more rare (11, 41). When chloroplast development is blocked by darkness, etioplasts are formed. In a fully developed etioplast, part of the membrane material is assembled in one or a few crystalline structures, PLBs, from which lamellar membranes extend into the stroma of the plastid (16). The lamellar membranes, the PTs, and the inner membrane of the envelope are frequently connected and it has been proposed that the etioplast could be regarded as a continuous membrane system (16, 39).

Isolated envelope fractions from etioplasts resemble envelope fractions from chloroplasts both concerning their lipid composition (1) and their polypeptide pattern (8) and it is reasonable to assume that UDP-galactos:1,2-diacylglycerol galactosyltransferase activity is associated with the etioplast envelope. We have investigated whether the galactosyltransferase activity is restricted to the envelope fraction of the etioplast, or to which extent this activity is present also in the PT and PLB fractions of this plastid.

MATERIALS AND METHODS

Plant Material. Grains of wheat (*Triticum aestivum* L. var Starke II; Weibull, Sweden) were soaked overnight in aerated tap water at 20°C and grown in darkness at 20°C, 100% RH, in a fertilized mixture of peat and sand (Hasselfors AB, Sweden) for 7 d. Three cm long leaf segments, cut 1.5 cm below the tip of the leaves, were used for the isolation of plastids. The harvest was done under dim green light at 4°C.

Isolation of Purified Etioplasts. Approximately 25 g of leaf tissue was homogenized in 210 ml of isolation medium (0.50 M sucrose, 10 mM Hepes, 20 mM Tes, pH 7.6/KOH) in a 0.5-L Turmix blender for 2 × 5 s at full speed. The homogenate was filtered through eight layers of cotton gauze and one layer of nylon cloth (mesh size 15 μm and the residue was rinsed with 2 × 20 ml of isolation medium. The filtrate was centrifuged at $1000g_{max}$ for 6 min in a fixed angle rotor (Sorvall, SS-34). The crude etioplast pellets were resuspended in 15 ml of isolation medium and loaded on a discontinuous gradient made of Percoll in isolation medium (15 ml of 25%, v/v, on top of 67%, v/v). After centrifugation at $1000g_{max}$ (Sorvall, swinging bucket rotor HB-4) for 20 min, etioplasts were collected from the interface between 25 and 67% Percoll. The etioplasts were washed with isolation medium 3 × 15 min at $1000g_{max}$ (Sorvall, HB-4 rotor).

Isolation of Envelope Membranes. Purified etioplasts from approximately 200 g of dark-grown wheat leaves were used for isolation of envelope membranes on a discontinuous sucrose gradient as described by Douce and Joyard (12).

Isolation of Prolamellar Bodies and Prothylakoids. The procedure, which is partly based on procedures described by Wellburn (40), Lütz (29), and Ryberg and Sundqvist (35), is summarized in Figure 1. A pellet of purified etioplasts from 100 to 150 g of leaf tissue was resuspended in 0.5 ml of 0.50 M sucrose in suspension buffer (10 mM Hepes, 20 mM Tes, 1.0 mM $MgCl_2$, 1.0 mM EDTA, pH 7.6/KOH) and diluted to 0.05 M sucrose with addition of suspension buffer. After 10 up-and-down strokes in a Ten-Broek glass and Teflon homogenizer, the suspension was centrifuged at $7700g_{max}$ (Sorvall, SS-34 rotor) for 15 min.

[1] Supported by the Swedish Natural Science Research Council.

[2] Abbreviations: MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol; PLB, prolamellar body; PT, prothylakoid.

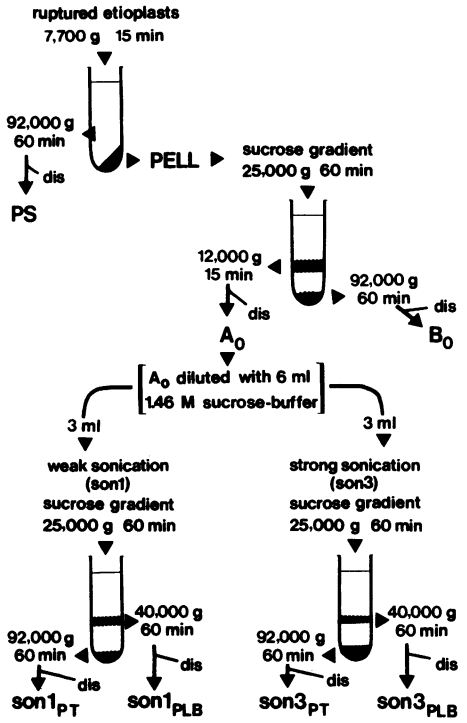

FIG. 1. Isolation of membrane fractions from purified etioplasts, isolated from dark-grown wheat leaves. dis, Discarded supernatant.

The supernatant (containing mainly membrane vesicles) was pelleted at 92,500g_{max} for 60 min in a fixed angle rotor (Beckmann, 70 Ti). The 7700g pellet, PELL (containing PLBs and membrane vesicles), was resuspended in 6 ml of 1.46 M sucrose in suspension buffer and divided into four aliquots that each was loaded at the bottom of a continuous sucrose gradient, made from equal volumes of 0.50 M sucrose and 1.40 M sucrose in suspension buffer. The gradients were centrifuged at 25,000g_{max} (Sorvall, SS-34 rotor) for 60 min. A band approximately 2.5 cm from the bottom, A_0 (containing mainly PLBs with attached membrane vesicles), and the bottom 2 to 3 ml, B_0 (containing mainly membrane vesicles), were separately removed. The pooled B_0 fractions were pelleted at 92,000g_{max} (Beckman, 70 Ti rotor) for 60 min. The pooled A_0 fractions were pelleted at 12,000g_{max} (Sorvall, SS-34 rotor) for 15 min, resuspended in 6 ml of 1.46 M sucrose in suspension buffer, and sonicated in a Branson Sonifer B-30, equipped with a microtip. Three ml of the suspension was sonicated for 3 × 5 s with the output control set at 1 (son1, weaker force) and 3 ml for 3 × 5 s with the output control set at 3 (son3, stronger force). The sonications were done in round-bottomed test tubes (i.d., 15.4 mm). Each sonicated suspension was loaded at the bottom of two sucrose gradients and centrifuged as described above. The PLB fractions, $son1_{PLB}$ and $son3_{PLB}$, were collected as described for A_0 and separately pelleted at 40,000g_{max} (Sorvall, SS-34 rotor) for 60 min. The membrane vesicle fractions, $son1_{PT}$ and $son3_{PT}$, were collected and pelleted as described for B_0. Pelleted fractions were resuspended in known volumes of assay medium (0.30 M sucrose, 10 mM Tricine, 4.0 mM $MgCl_2$, pH 7.6/KOH) and samples were removed for analyses as described below. In cases of SDS-PAGE, the membrane fractions were suspended in sucrose-free medium.

Except for sucrose gradient runs, suspensions of subplastid fractions were always diluted to 0.5 M sucrose before centrifugation. All steps in the isolations of etioplasts and subplastid fractions were carried out under dim green light at 0 to 4°C.

Galactolipid Biosynthesis. Fifty μl of suspended subplastid fraction (containing up to 300 μg protein) was added to 360 μl of assay medium containing 0.50 mM UDP-galactose and 37·10^3 Bq UDP-[^{14}C]galactose. After different incubation times at 25°C, aliquots were removed and added to 1-ml portions of chloroform:methanol 1:2 (v/v). The lipids were extracted according to Bligh and Dyer (3) and separated by TLC on silica gel plates (0.5 mm, made from Merck Silica gel 60H) with the solvent system chloroform:methanol:acetic acid: water (85:15:10:3.5, by volume [31]). In this system, UDP-galactose remained at the application point. After development, the plates were exposed to iodine vapor to locate MGDG and DGDG. When the iodine had evaporated, silica gel areas containing these lipids were separately scraped off the plates into counting vials, 10 ml of scintillation cocktail (Packard Insta-Gel II) were added, and the radioactivity was monitored in a Packard Tri-Carb 460 CD Liquid Scintillation system.

Lipid Analyses. Lipids were extracted (3) and the acyl groups of the lipids were methylated with 14% BF_3 in methanol (30). The methyl esters of the acyl groups were separated by GC on a 0.13- (i.d.) × 180-cm glass column packed with 10% SP-2330 on Chromosorb W/AW 100/120 mesh (Supelco) in a temperature program from 140 to 185°C. The flow of carrier gas, N_2, was 20 ml min^{-1}. A Varian 3200 gas chromatograph with a flame ionization detector was used. Heptadecanoic acid methyl ester was used as internal standard and a Hewlett-Packard 3390 integrator was used for quantification.

Pigment Analyses. Pigments were extracted with acetone:water 85:15 (v/v) and PChlide was determined according to Koski and Smith (23). After addition of water to the pigment extract, carotenoids were extracted with petrol fraction (bp 40–60°C). Total carotenoids, dissolved in petrol fraction (bp 40–60°C):acetone (98:2, v/v), were determined spectrophotometrically at 440 nm using the extinction coefficient $\epsilon = 253\ g^{-1}\ cm^{-1}$.

Protein Analyses. Proteins were solubilized in 0.08 M KOH at 4°C overnight and determined by a color reaction using Coomassie Brilliant Blue G-250 (33). Bovine serum albumin (essentially fatty acid free) in assay medium with 0.08 M KOH was used as standard.

SDS-PAGE. Polypeptides, precipitated from the different etioplast membrane fractions with acetone, were separated by SDS-PAGE according to Laemmli (24). The stacking gel was made of 5% (w/v) acrylamide. The separating gel (0.8 mm thick, 18 cm long) was made of a linear gradient of acrylamide (10–20%, w/v) and glycerol (1.5–6.5%, v/v) or of 12% acrylamide. The proteins of the membrane fractions were solubilized for 30 min at 40°C in a SDS-buffer with a SDS to protein ratio of 7.5:1 (w/v). After separation of the polypeptides, the gels were fixed for 2 h in acetic acid:methanol:water (5:20:75, by volume), stained for 2 h with 0.04% (w/v) Coomassie Brilliant Blue G-250 in $HClO_4$:methanol:water (3.5:20:76.5, by volume), and destained in acetic acid:water (5:95, v/v).

Mol wt of the major polypeptide bands were estimated by comparing R_F values with those of reference proteins: phosphorylase *b* (mol wt 94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400; LMV Calibration Kit, Pharmacia Fine Chemicals, Sweden).

Electron Microscopy. Isolated plastids and subplastid fractions were fixed in glutaraldehyde, embedded in agar, and postfixed in OsO_4 as described by Ryberg and Sundqvist (35) and further prepared for electron microscopy as described by Sundqvist and Ryberg (37).

All chemicals were of analytical grade. BF_3 (14%) in methanol and UDP-galactose were obtained from Sigma; Coomassie Brilliant Blue G-250 from Serva, F.R.G; methyl heptadecanoate from Supelco Inc.: Percoll (density 1.129 g ml^{-1}) from Pharmacia Fine Chemicals, Sweden; and UDP-[^{14}C]galactose (11.43 × 10^{12} Bq mol^{-1}) from The Radiochemical Center, Amersham, England. Chloroform, methanol, and petrol fraction (bp 40–60°C) were distilled, and acetic acid, acetone, and heptane were pro analysis (Merck, F.R.G.).

RESULTS

Electron micrographs of purified etioplast fractions revealed that the presence of mitochondria was negligible (Fig. 2). The purified etioplasts represented 1 to 3% of the plastids of the leaf segments used. This estimation is based on carotenoid measurements.

Galactolipid Biosynthesis. Different subplastid membrane fractions were isolated from osmotically and mechanically ruptured etioplasts. The isolation procedure is presented in Figure 1 and electron micrographs of the fractions obtained are presented in Figure 3. Each step of the isolation procedure resulted in a 10 to 50% loss of material as measured by carotenoid content.

The first step removed a fraction of membrane vesicles (PS fraction, containing most of the envelope material) from the inner membranes of the etioplasts (PELL fraction). The distribution of UDP-galactose galactosyltransferase activity between the PS and PELL fractions is shown in Table I, and time course studies of UDP-galactose incorporation into the fractions are shown in Figure 4.

The PELL fraction was loaded at the bottom of a continuous sucrose gradient. After centrifugation, membrane vesicles were found at the bottom of the gradient (fraction B_0) while the osmotically nonactive PLBs had moved up the gradient during the centrifugation. This PLB fraction (A_0) also contained membrane vesicles, probably connected with the PLBs. UDP-galactose galactosyltransferase activity was present in both fractions (A_0, B_0), derived from the PELL fraction (Table II; Fig. 5a). PChlide was detected in all fractions (PS, PELL, A_0, B_0).

The A_0 fraction, containing PLBs with attached PTs, was sonicated with the purpose of separating the PTs from the PLBs. Two forces of sonication were used. The weaker force (son1) released a portion of the membrane vesicles (PTs) from the PLBs without fragmentating the PLBs to any greater extent. The stronger force of sonication (son3) resulted in a PLB fraction with few membrane vesicles, while the membrane vesicle fraction obtained was heavily contaminated with PLB fragments. Thus, weak sonication force yielded the least contaminated PT fraction ($son1_{PT}$, Fig. 3e), while strong sonication force yielded the purest PLB fraction ($son3_{PLB}$, Fig. 3f). UDP-galactose galactosyltransferase activity was present in all fractions obtained from sonicated A_0 fraction (Fig. 5, b and c). The initial linear rate of MGDG synthesis was lowest in the fractions enriched in PLBs ($son1_{PLB}$, $son3_{PLB}$). The total MGDG synthesis during the linear stage was

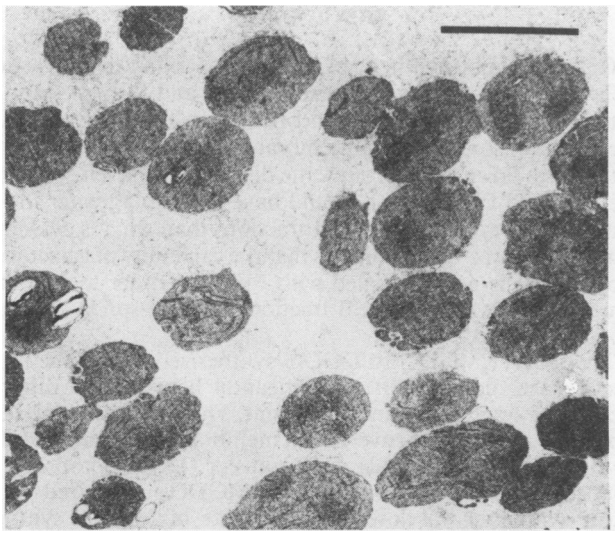

FIG. 2. Electron micrograph of a fraction of purified etioplasts, isolated from dark-grown wheat leaves. The bar represents 3 μm.

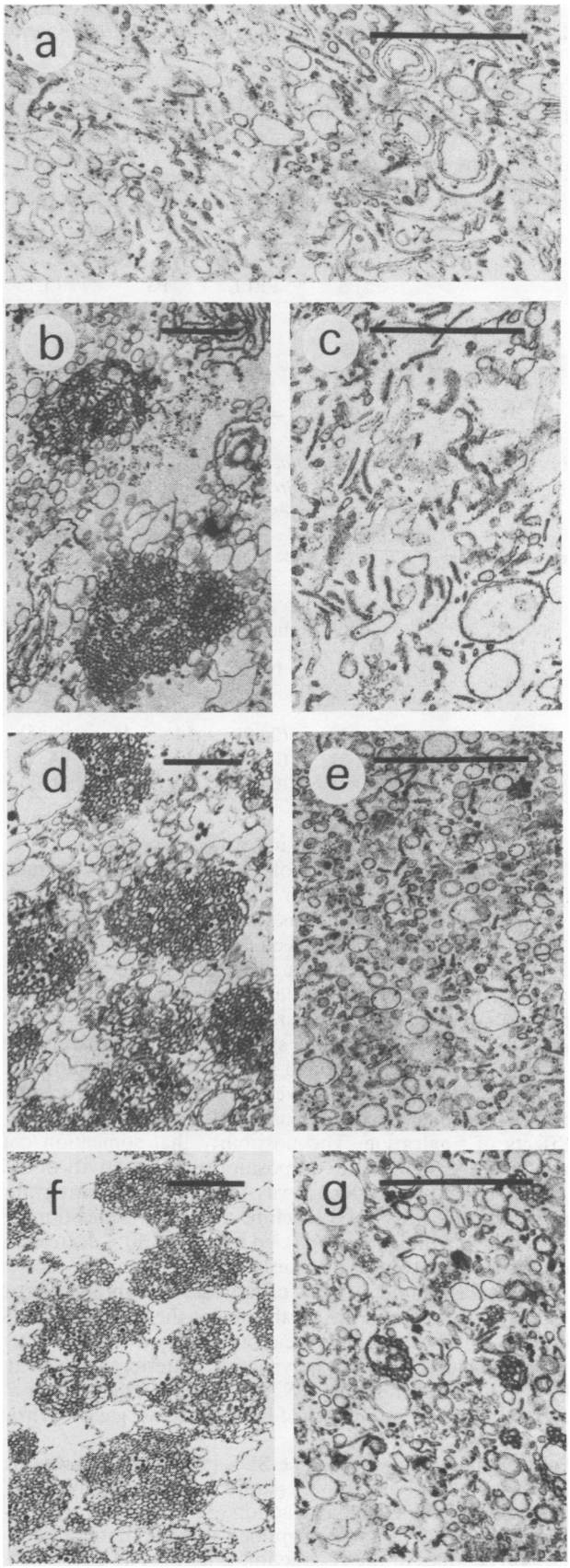

FIG. 3. Electron micrographs of membrane fractions, isolated from dark-grown wheat leaves. (a) PS fraction; (b) A_0 fraction; (c) B_0 fraction; (d) $son1_{PLB}$ fraction; (e) $son1_{PT}$ fraction; (f) $son3_{PLB}$ fraction; (g) $son3_{PT}$ fraction. For an explanation of abbreviations, see Figure 1. The bars represent 1 μm.

Table I. *Distribution of UDP-Galactosyl Transferase Activity and Proteins between the PS and PELL Fractions Isolated from Etioplasts of Dark-Grown Wheat*

UDP-galactosyl transferase activity was measured as the initial, linear rate of incorporation of ^{14}C from UDP-[^{14}C]galactose into MGDG. Results from three independent experiments are shown. For explanation of abbreviations, see Figures 1 and 3.

	Distribution of MGDG synthesis		Distribution of Proteins	
	PS	PELL	PS	PELL
	%			
Exp I	45	52	26	74
Exp II	47	53	22	78
Exp III	48	52	28	72

Table II. *Distribution of UDP-Galactosyl Transferase Activity between the A_0 and B_0 Fractions Derived from a Fraction of Etioplast Inner Membranes (PELL)*

rec, recovery = $\frac{A_0 + B_0}{PELL}$, otherwise as in Table I.

	Distribution of MGDG Synthesis			Distribution of Proteins		
	A_0	B_0	(rec)	A_0	B_0	(rec)
	%					
Exp I	52	48	(0.65)	54	46	(0.85)
Exp II	19	81	(0.70)	47	53	(0.35)
Exp III	12	88	(0.90)	47	53	(0.85)

lower in $son3_{PLB} + son3_{PT}$ than in $son1_{PLB} + son1_{PT}$.

In all membrane fractions of all experiments, the rate of MGDG synthesis was linear for less than 2.5 min (Figs. 4 and 5). The rate of DGDG synthesis was linear for between 10 and 60 min, the time differing between different experiments.

Between 95% and 98% of the radioactivity incorporated into galactolipids from UDP-[^{14}C]galactose during the 1st min of the assay was found in MGDG. The remainder was found in DGDG. The ratio of MGDG to DGDG synthesized was similar between all membrane fractions obtained from an individual subfractionation of etioplasts. No radioactive trigalactosyl diacylglycerol and tetragalactosyl diacylglycerol could be detected.

Effects of Sonication. The possibility that sonication could have altered the membrane composition and the activity of UDP-galactose galactosyltransferase was tested by comparing membrane fractions (PS, A_0, B_0) before and after sonication. The strong force of sonication (son3) led to a 20 to 30% decrease and the weak force of sonication (son1) to a less than 10% decrease in the protein to lipid content of lamellar membrane fractions (PS, B_0), while this ratio was unaffected in the mainly crystalline fraction (A_0). The total fatty acid composition was not altered by sonication, nor were the polypeptide patterns as analyzed by SDS-PAGE. In all fractions (PS, A_0, B_0) sonication led to a 30 to 60% decrease in the activity of UDP-galactose galactosyltransferase.

Polypeptide Composition. The SDS-PAGE of the proteins of etioplast membrane fractions separated approximately 35 to 40 polypeptides of the envelope fraction, 30 to 35 polypeptides of the PS fraction, 20 to 25 polypeptides of the B_0 fraction, and 15 to 20 polypeptides of the A_0 fraction (Fig. 6). The envelope and PS fractions had five major polypeptides in common. These had mol wt of 51, 36, 29, 24, and $16 \cdot 10^3$, respectively. Other prominent bands in the envelope fraction had mol wt of 114, 65, 42, and $32 \cdot 10^3$ and, in the PS fraction, 100, 56, 33, and $18 \cdot 10^3$. The

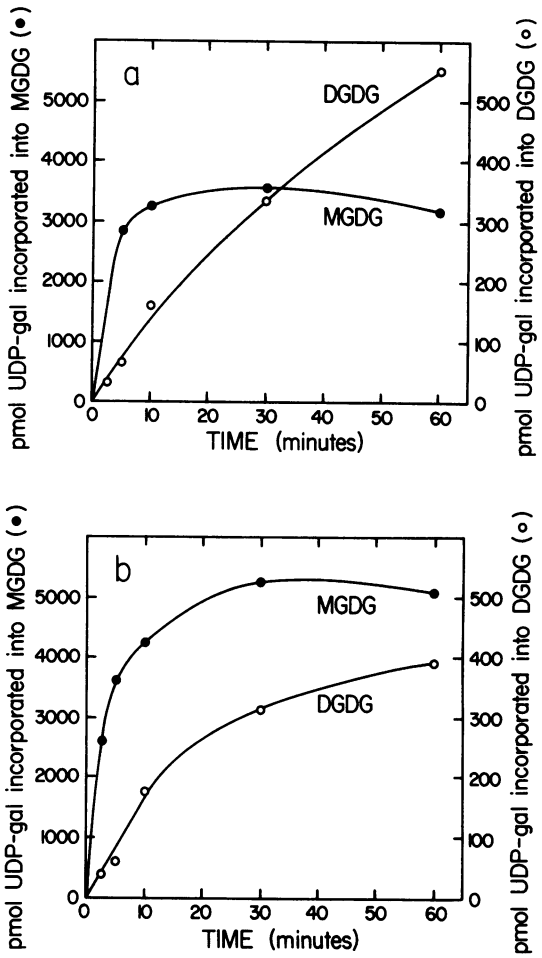

FIG. 4. Time course studies of UDP-galactose (UDP-gal) incorporation into MGDG (●) and DGDG (○) of (a) the PS fraction and (b) the PELL fraction, isolated from etioplasts of dark-grown wheat leaves. For an explanation of abbreviations, see Figures 1 and 3.

contribution of the 51 and $16 \cdot 10^3$ bands varied between different isolations. The major polypeptides of the A_0 and B_0 fractions had mol wt of 56 and $35 \cdot 10^3$. Some of the minor polypeptides of the A_0 and B_0 fractions had similar mol wt to polypeptides of the envelope and PS fractions.

DISCUSSION

Galactolipid biosynthetic capability was found in all fractions isolated from etioplasts of wheat (Tables I and II; Figs. 4 and 5). The activity was lower in the PLB than in the PT fractions. If the effects of sonication on membrane composition and enzyme activity are taken into account, the difference in activity between the PLB and PT fractions should have been still greater for the son3 fractions than for the son1 fractions than what is evident in Figure 5, b and c. This indicates that the capability of galactolipid biosynthesis is not associated with PLBs. In order to settle this issue, however, a purer PLB fraction than the $son3_{PLB}$ fraction would be needed.

The ratio of MGDG to DGDG synthesized during the initial linear stage did not differ significantly between the different membrane fractions of an experiment. This shows that the same enzyme system was active in all membrane fractions. DGDG synthesis by interlipid galactosyl transfer (18, 38) probably did not occur since the ratio of DGDG to MGDG synthesized (initial linear synthesis) was low. Also, the rate of MGDG synthesis declined after only a few minutes (Figs. 4 and 5) while the

DGDG synthesis rate remained linear for 10 to 60 min. The decline in MGDG synthesis was probably due to lack of available diacylglycerol since less than 5% of the UDP-galactose adminis-

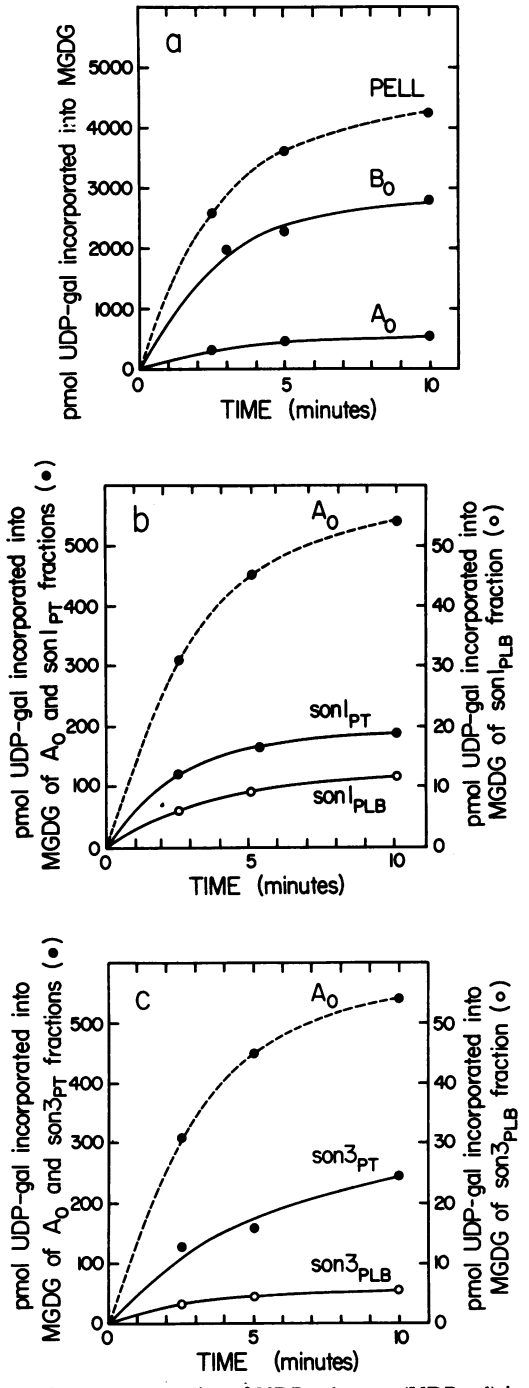

FIG. 5. Time course studies of UDP-galactose (UDP-gal) incorporation into MGDG of different subfractions of inner membranes of etioplasts of dark-grown wheat. (a) The A_0 and B_0 fractions derived from the PELL fraction; (b) the $sonI_{PLB}$ and $sonI_{PT}$ fractions derived from an aliquot of the A_0 fraction that had been subjected to a weak force of sonication; (c) the $son3_{PLB}$ and $son3_{PT}$ fractions derived from an aliquot of the A_0 fraction that had been subjected to a strong force of sonication. In a to c, data calculated for the whole A_0 fraction prior to sonication are shown. In b and c, the data for the fractions derived from the A_0 fraction have been calculated to show the recovery. For an explanation of abbreviations, see Figures 1 and 3. Figures 4 and 5 present data from the same experiment.

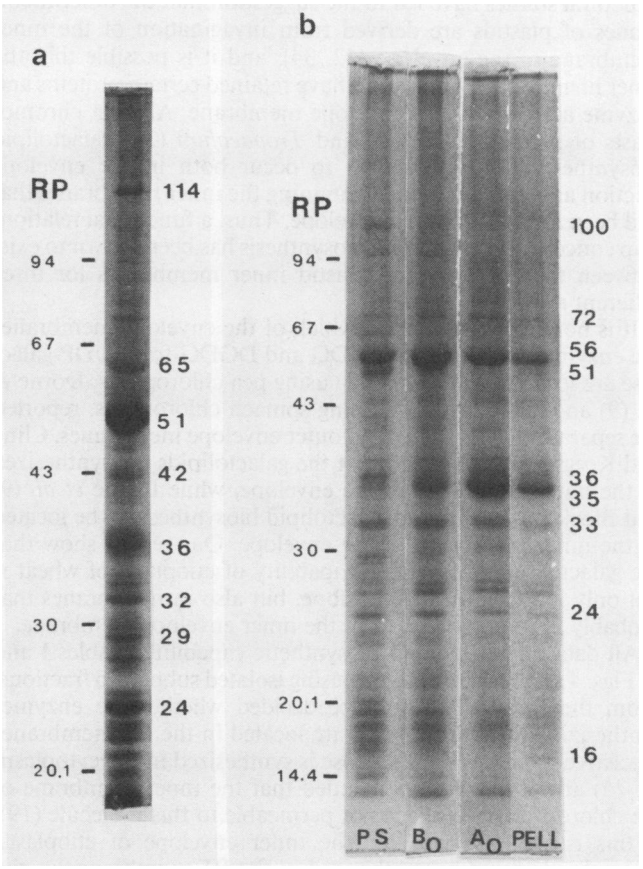

FIG. 6. Electrophoretic separation of acetone-extracted polypeptides of membrane fractions isolated from etioplasts of dark-grown wheat leaves. (a) Envelope fraction. The separating gel contained 12% (w/v) acrylamide. (b) PS, A_0, B_0, and PELL fractions. The separating gel was made from a linear gradient of acrylamide (10–20%, w/v). Mol wt of the reference proteins (RP) are indicated, as are the mol wt estimations of the major polypeptides of the fractions (mol wt $\times 10^{-3}$ are shown). For an explanation of abbreviations, see Figures 1 and 3.

tered was utilized.

Joyard *et al.* (22) showed that the polypeptide pattern of envelope membranes of spinach chloroplasts is very different from that of the stroma or the thylakoids. The envelope fraction of etioplasts (Fig. 6a) resembled chloroplast envelope fractions of spinach (22, 36) and pea (7) in containing some polypeptides of mol wt over $100 \cdot 10^3$ and in having polypeptides of mol wt of 72, 65, 50–54 (ribulose-1,5-bisphosphate carboxylase, large subunit [36]), 42, 36, 29 (phosphate translocator [14]), and $24 \cdot 10^3$. The fraction of etioplast envelopes differed from chloroplast ones (7, 22, 36) in having polypeptides of mol wt between 14 and $24 \cdot 10^3$ and in that phosphate translocator was not one of the major polypeptides. The PS fraction (Fig. 6b) contained most of the polypeptides present in the envelope fraction, together with some polypeptides of inner membrane origin, as those of mol wt of 56 and $33 \cdot 10^3$. The polypeptide pattern of the PELL fraction and of the A_0 and B_0 fractions derived from the PELL fraction were dominated by the 56 and $35 \cdot 10^3$ polypeptides (Fig. 6b). The $56 \cdot 10^3$ polypeptide was found in the envelope fraction, but not the $35 \cdot 10^3$ one. Some polypeptides were common between the envelope fraction (Fig. 6a, and the A_0 and B_0 fractions (Fig. 6b).

The findings of polypeptides common between an envelope fraction and the inner membranes of etioplasts and the presence of galactolipid biosynthetic capability in all membrane fractions of etioplasts could be caused by the presence of envelope vesicles in the different etioplast membrane fractions. However, ultra-

structural studies have led to the suggestion that the inner membranes of plastids are derived from invagination of the inner membrane of the envelope (12, 34), and it is possible that the inner membranes of etioplasts have retained certain proteins and enzyme activities of the envelope membrane. Also, in chromoplasts of *Narcissus* (26, 27) and *Tropaeolum* (28) galactolipid biosynthesis has been shown to occur both in the envelope fraction and in the fraction containing the inner membranes that had been formed from the envelope. Thus, a functional relationship concerning galactolipid biosynthesis has been shown to exist between the envelope and plastid inner membranes for three different non-green plastids.

It is not yet established in which of the envelope membranes the enzymes synthesizing MGDG and DGDG from UDP-galactose are located. Cline *et al.* (7) using pea chloroplasts, Dorne *et al.* (9) and Block *et al.* (4) using spinach chloroplasts, reported the separation of the inner and outer envelope membranes. Cline and Keegstra (6) proposed that the galactolipids are synthesized in the outer membrane of the envelope, while Dorne *et al.* (9) and Block *et al.* (5) found galactolipid biosynthesis to be located in the inner membrane of the envelope. Our results show that the galactolipid biosynthetic capability of etioplasts of wheat is not only localized in the envelope, but also in membranes that probably have originated from the inner envelope membrane.

All data on galactolipid biosynthetic capability (Tables I and II; Figs. 4 and 5) were obtained using isolated subplastid fractions. From these data, it cannot be decided whether the enzymes synthesizing galactolipids that are located in the PT membranes are active *in situ*. UDP-galactose is synthesized in the cytoplasm (2, 25) and it has been concluded that the inner membrane of the chloroplast envelope is not permeable to this molecule (19). If this is the case also for the inner envelope of etioplasts, galactolipids could be synthesized in the PT membranes *in situ* only if the inner envelope membrane and the PT membranes form a continuous membrane system.

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