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Short Communication

Lipid and Fatty Acid Composition of Chloroplast Envelope Membranes from Species with Differing Net Photosynthesis

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ABSTRACT

Lipid and fatty acid compositions were determined for chloroplast envelope membranes isolated from spinach (*Spinacia oleracea* L.), sunflower (*Helianthus annuus* L.), and maize (*Zea mays* L.) leaves. The lipid composition was similar in sunflower, spinach, and undifferentiated maize chloroplast envelope membranes and different in maize mesophyll chloroplast envelope membranes. The predominant lipid constituents in all envelope membranes were monogalactosyldiglyceride (27 to 46%), digalactosyldiglyceride (18 to 33%), and phosphatidylcholine (7 to 30%). The fatty acid composition was also similar in sunflower and spinach chloroplast envelope membranes in comparison to those from maize. The major acyl fatty acids of the chloroplast envelope membrane were palmitic ($C_{16:0}$, 41 and 36%) and linolenic ($C_{18:3}$, 29 and 40%) acids for spinach and sunflower; palmitic (77%) and stearic ($C_{18:0}$, 12%) acids for young maize; and palmitic (61%), stearic (14%), and linolenic (13%) acids for mature maize. The differences in lipid and acyl fatty acid compositions among these plants which vary in their rates of net photosynthesis were largely quantitative rather than qualitative.

Methods for isolating chloroplast envelope membranes from plant species with slow rates of net photosynthesis have been developed in several laboratories (4, 11, 17, 20). Recently I have also described the isolation of such membranes from plant species with fast rates of net photosynthesis (21). I believe that differences in the biochemical properties of these envelope membranes might be related to variations in chloroplastic permeability and metabolite transport and hence these variations in biochemical properties could effect photosynthetic efficiency (21).

Characterization of these membranes has shown some differences in their transport abilities (18, 19, 21), enzymic levels (21), and hexosamine contents (22). The lipid compositions were previously determined only with chloroplast envelope membranes from plant species with slow rates of net photosynthesis (4, 12, 17), hence comparative studies are incomplete. This paper describes differences among the lipid and acyl fatty acid compositions of spinach, sunflower, and maize chloroplast envelope membranes. These plant species were selected for investigation because of their known differences in rates of net photosynthesis at high irradiance in normal air.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Spinach (*Spinacia oleracea* L., var. Viroflay, Asgrow Seed Co.), maize (*Zea mays* L., hybrid 595 S, Agway Inc.), and sunflower (*Helianthus annuus* L., Hybrid 896 [cmsHA 89 × RHA 266], a gift of G. Fick, United States Department of Agriculture at North Dakota State University) were grown in Vermiculite in the greenhouse maintained at day/night temperatures of 22/16 C. Nutrient solution was added twice weekly.

Chloroplasts. Intact mesophyll chloroplasts were isolated from 10-g batches of freshly harvested, rinsed leaves 4 to 6 weeks of age for preparation from spinach, sunflower, and mature maize (5, 8, 25). Undifferentiated intact chloroplasts were prepared from 4- to 6-day-old leaves of young maize (15).

Chloroplast Envelope Membranes. Complete envelope membranes were removed from intact spinach, sunflower, and maize chloroplasts by osmotic shock and purified on a three phase discontinuous sucrose gradient. Details of the isolation procedure were described previously (21). Complete envelope membrane fractions were pooled and diluted 2-fold with 330 mM sorbitol in 50 mM Tricine at pH 7.6. After centrifugation at 75,000*g* for 60 min, the supernatant fluid was carefully removed and the pellet was used directly for lipid extraction.

Lipid Extraction. Lipids of purified envelope membranes were extracted in a Ten-Broeck homogenizer successively with chloroform, methanol, and water by Bligh and Dyer procedure (3). Lipids were extracted and concentrated at 4 C under N_2. Dry weights of aliquots were determined after drying at 65 C.

Lipid Composition. Lipids were resolved by two-dimensional TLC Silica Gel G plates (250 μm × 20 cm^2) containing no gypsum or fluorescent indicator (Brinkmann Instruments, Inc.). Plates were activated at 110 C. Development was first carried out in chloroform-methanol-acetic acid-water (170:25:25:6) and then in chloroform-methanol-7 N NH_4OH (65:30:4). Plates were dried between changes in solvent systems in a stream of N_2 at room temperature for 20 min to minimize lipid breakdown on the plate. After development, plates were oven-dried at 50 C for 1 hr. This procedure gave excellent separations of glycolipids and phospholipids. Neutral lipids were resolved by one-dimensional TLC in hexane-diethyl ether-acetic acid (80:20:1). Procedures were as described previously (17).

Compounds were identified by several spray reagents (17) and comparison with R_F values obtained with lipid standards (Supelco, Inc.). For lipid analyses compound locations were revealed by iodine vapor and each lipid was carefully removed by scraping off the silica gel with a razor blade after the iodine was evaporated.

Glycolipids were determined in the presence of adsorbent with the phenol-sulfuric acid colorimetric procedure of Roughan and Batt (23). Sterol glycoside and acylated sterol glycoside were acid-hydrolyzed and the liberated sugar was quantitatively determined after extraction from the sterols, since the steryl moiety gave rise to color interference. Phospholipids were measured

colorimetrically in the presence of adsorbent by the molybdate method of Rouser *et al.* (24). Color formation by sterols with the phenol-sulfuric reagent in the presence of adsorbent was linear with concentration, and was used for the determination of sterols and steryl esters. Other neutral lipids were not subjected to rigorous quantitative analyses, but spot sizes were compared to known amounts produced with standards. Chl was determined in 80% acetone.

Fatty Acid Composition. Lipids were saponified in 0.5 N methanolic NaOH and esterified (14) with 10% BCl_3 in methanol (esterification kit, Applied Science). A reverse phase partition system of TLC was used for supplementary identification of the fatty acid methyl esters. Silica Gel G plates impregnated with silicone oil were developed in acetonitrile-glacial acetic acid-water (70:10:25) and fatty acid methyl esters were located with sulfuric acid-dichromate spray (13) and compared with R_F values of standards (Applied Science).

The fatty acid methyl ester composition was determined by gas chromatography on a Bendix 2600 gas chromatograph equipped with glass columns (i.d. 2 mm) containing 12% diethylene glycol succinate (stabilized) on 100/110 mesh Anakrom Q (Analabs) at 170 C.

RESULTS AND DISCUSSION

Similarities in the lipid compositions of chloroplast envelope membranes, which are predominately double membrane vesicles (21) isolated from spinach, sunflower, and maize, are shown in Table I. The glycolipids are the major lipids, and they are composed mainly of monogalactosyldiglyceride and digalactosyldiglyceride. Phospholipids are present in a lesser amount, and the major lipid in this group is phosphatidylcholine.

The lipid composition of envelope membranes from undifferentiated maize chloroplasts is very similar to chloroplasts of plants lacking bundle-sheath chloroplasts (Table I). However, changes in the lipid composition of the envelope membrane occur as the undifferentiated form of the maize chloroplast develops into its mature forms, the mesophyll and bundle-sheath chloroplasts (16). The envelope membrane of maize mesophyll chloroplasts contains more monogalactosyldiglyceride, sterylglycoside, and acylated sterylglycoside and less digalactosyldiglyceride and phosphatidylcholine than its counterpart in plants containing no bundle-sheath chloroplasts. The carbon reduction pathways in mesophyll chloroplasts of plants without bundle-sheath chloroplasts are similar to that in undifferentiated maize chloroplasts (15). It is only after differentiation into the two chloroplast types that the C reduction pathways diverge (2). Whether changes in overall lipid composition are related to changes in membrane permeability or C reduction pathways is presently unknown.

Lipid compositions have been described previously for chloroplast envelopes of spinach (4, 17) and broad bean (12). Generally the trends are the same when compared with Table I. Some variations exist in the phosphatidylcholine content in different preparations, although it is always the major phospholipid. Freshly isolated chloroplast envelopes appear to give higher values for phosphatidylcholine (12) and variations in purity and extent of envelope membranes containing the complete structure (double membrane as opposed to single) may introduce further changes in the phosphatidylcholine content. Lipid compositions in Table I are for chloroplast envelope membranes with a predominantly complete (double membrane) structure (21).

Examination of the lipid compositions of lamellar membranes in spinach and maize mesophyll chloroplasts (1, 9) compared with those of the envelope membranes reveals several differences. Both contain glycolipids and phospholipids as major and minor lipids, respectively. However, more monogalactosyldyglyceride, digalactosyldiglyceride, and phosphatidylcholine and less sulfolipid and phosphatidylglycerol are found in the envelope membranes compared with the lamellar membranes. Other lipids (cerebroside, sterylglycoside, acylated sterylglycoside, phosphatidylethanolamine, sterol, and steryl ester) are present exclusively in the envelope membranes. Undoubtedly these differences in lipid composition reflect the differences of function between the envelope and lamellar membranes.

The fatty acid compositions of the various chloroplast envelope membranes showed greater variation (Table II) among plant species than the overall lipid compositions. In spinach and sunflower membranes the major acyl fatty acids were palmitic (16:0) and linolenic (18:3) acids. Sunflower envelope membrane fatty acids showed a higher degree of unsaturation than spinach (Table II). Undifferentiated maize chloroplast envelope mem-

Table I. *Lipid Composition of Isolated Chloroplast Envelope Membranes*

	Spinach	Sunflower	Maize	
			Undifferentiated	Mesophyll
		Dry wt % of total lipid		
Monogalactosyldiglyceride	27.1	31.0	34.0	46.3
Digalactosyldiglyceride	33.1	25.5	24.0	18.2
Trigalactosyldiglyceride	1.4	0.3	0.3	0.2
Sulfolipid	0.1	0.7	0.4	2.9
Cerebroside	0.4	0.1	tr	1.9
Sterylglycoside	0.9	1.6	0.3	3.8
Acylated sterylglycoside	1.8	1.0	0.3	4.6
Sterol	1.9	0.9	tr	0.8
Steryl ester	1.8	1.8	0.9	1.5
Phosphatidylcholine	25.1	28.9	29.9	6.7
Phosphatidylglycerol	6.2	5.3	4.0	2.2
Phosphatidylethanolamine	1.4	1.8	1.4	1.1
Phosphatidylinositol	0.6	0.6	0.7	1.4
Diphosphatidylglycerol	tr[a]	0	0	0.8
Chlorophyll	tr	tr	1.0	0.7
Carotenoid	+[b]	+	+	+
Remaining Neutral Lipid				
Quinone	+	+	0	+
Fatty acid	+	+	0	+
Fatty acid methyl ester	+	+	0	+
Triglyceride	+	0	0	+
mg total lipid/mg protein	1.0	1.2	1.4	1.3

[a] Trace.

[b] Present in undetermined, probably trace amounts.

Table II. *Acyl Fatty Acid Composition of Isolated Chloroplast Envelope Membranes*

	Spinach	Sunflower	Maize	
			Undifferentiated	Mesophyll
		Wt, % of Total Fatty Acids		
< 16:0[a]	tr[b]	tr	1.40	tr
16:0	41.4	35.9	77.3	61.4
16:1	3.4	2.6	tr	0
16:3	2.4	0.6	5.4	3.4
18:0	10.5	4.9	11.7	13.7
18:1	4.6	1.9	1.0	1.8
18:2	8.4	13.5	1.5	3.8
18:3	28.9	39.6	0.4	13.2
20:0	tr	tr	0.8	2.2
unsaturated/saturated	0.9	1.4	0.1	0.3

[a] Primarily 14:0 in saturated and unsaturated forms.
[b] Trace.

branes are very rich in palmitic, and to a much lesser degree, stearic acid and very low in unsaturated fatty acids. Since fatty acid biosynthesis in higher plants proceeds from *de novo* production of palmitate followed by addition of C_2 units in conjunction with monenoic and polyenoic desaturation (7), high levels of palmitate are not unexpected considering the very young age of the leaf material (4 to 6 days). Envelope membranes from maize mesophyll chloroplasts do show an increase in longer chain and unsaturated fatty acids. However, the amounts are smaller than those in plants lacking bundle-sheath chloroplasts.

Acyl fatty acid compositions of complete envelope membranes shown here (Table II) differ from those previously given for envelope membranes of spinach (4) and broad bean chloroplasts (12) in one aspect. The amounts of palmitic acid shown in Table II are higher and the linolenic acid contents are lower. It is difficult to ascertain whether these differences arise from variations in plant species, age, growing conditions, purity of preparation, or the extent of double membrane structure.

The acyl fatty acids of chloroplast envelope membranes from spinach are more saturated and have a lower linolenic to palmitic acid ratio (Table II) than do the chloroplast lamellae (1). Similar results were described for the acyl fatty acids of broad bean chloroplast lamellae and envelope membranes (12). Envelope membranes of undifferentiated and mesophyll maize chloroplasts have higher levels of saturated acyl fatty acids than do spinach envelope membranes (Table II). Increasing amounts of saturated fatty acids in membranes result in a higher temperature of phase transition (6), but the effect upon membrane permeability is still uncertain. On the basis of their increased fatty acid saturation, the envelope membranes should have greater thermostability than the lamellae, and the maize envelope membranes should be more thermostable than those of spinach. Recent evidence with spinach (10) in fact shows that the envelope membrane has greater thermostability than the lamellae. At 35 C the rate of net photosynthesis for maize in normal air under high illumination is three times greater than spinach (26). Whether this is in part related to thermostability and membrane integrity is uncertain.

The differences in lipid and fatty acid compositions of the chloroplast envelope membranes of species that vary in photosynthetic rate are thus largely quantitative rather than qualitative in nature.

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