Lipid composition of chloroplasts isolated by aqueous and nonaqueous techniques

ALPASLAN ONGUN, W. W. THOMSON, and J. B. MUDD

Department of Biochemistry, Department of Life Sciences, and Statewide Air Pollution Research Center, University of California, Riverside, California 92502

ABSTRACT Chloroplasts isolated from tobacco leaves in 0.5 M sucrose solution (the 1000 *g* pellet) contained 83% of the total cellular monogalactosyl diglyceride, 88% of the digalactosyl diglyceride, 76% of the sulfolipid, and 74% of the phosphatidyl glycerol. Phosphatidyl inositol was concentrated in the 15,000 *g* pellet. Phosphatidyl choline and phosphatidyl ethanolamine were concentrated in the 15,000 *g* supernatant fraction.

Chloroplasts isolated from tobacco leaves by a nonaqueous technique in hexane–carbon tetrachloride show a glycerolipid composition similar to that found in chloroplasts isolated in the aqueous system, even though some lipid, particularly monogalactosyl diglyceride, is extracted by the organic solvent during the process.

KEY WORDS glycerolipids · distribution · cell fractions · tobacco leaves · chloroplast · nonaqueous isolation · electron microscopy · grana

THE LOSS OF WATER-SOLUBLE COMPOUNDS from chloroplasts during isolation by differential centrifugation can be prevented by using organic solvents in the fractionation of lyophilized leaves (1–3). The nonaqueous method has overcome some inherent disadvantages of the aqueous technique. It has been successfully used to demonstrate the subcellular distribution of metal ions (4), the localization and migration of the early products of photosynthesis (5, 6), and the coenzyme content of chloroplasts (7), and has provided evidence of the localization of enzymic activities (8, 9).

While the nonaqueous technique has the advantage of retaining water-soluble compounds in the chloroplast, it may be expected to dissolve some of the lipid components and so would probably be unsuitable for demonstrating the localization of lipophilic intermediates of metabolism. It is also unlikely that the nonaqueous technique would be suitable for demonstrating the localization of enzymes which either act in a lipoprotein environment or require lipid cofactors. Thalacker and Behrens have shown (10) that organic solvents used in the nonaqueous isolation of chloroplasts extract 10% of the lipid phosphorus, 11% of the lipid nitrogen, and 2% of the chlorophyll. It has also been shown that organic solvent extraction of chloroplasts removes the quinones necessary for photoreductive reactions (11, 12).

In the course of experiments on the synthesis of fatty acids and complex lipids by chloroplasts and algae (13, 14) we have considered the use of the nonaqueous technique for obtaining enzymically active preparations. We have, therefore, determined the extent to which the composition of glycerolipids is changed during chloroplast isolation in organic solvents.

EXPERIMENTAL PROCEDURE

Greenhouse-grown tobacco plants (*Nicotiana tobacum* var. Mammoth gold) were used. Mature leaves were harvested from 2-month old plants immediately after their removal from the greenhouse.

For the aqueous fractionation, midribs and large lateral veins of washed leaves were removed, and 60 g of leaf tissue was ground in a Waring Blendor in about 80 ml of 0.5 M cold sucrose solution for a total of 1.5 min, with a short stop after each 30 sec. The homogenate was filtered through four layers of cheesecloth and the filtrate was fractionated by differential centrifugation. Five fractions, including the original filtrate, were obtained.

A portion of the filtrate was centrifuged at 200 *g* for 2 min and the resulting pellet was saved. This pellet con-

tained the unbroken cells, nuclei, and a small amount of chloroplasts. The supernatant solution was centrifuged at 1000 *g* for 7 min to give the chloroplasts. The resulting supernatant solution was recentrifuged at 15,000 *g* for 15 min, which gave a pellet (containing the mitochondria and large amounts of broken chloroplasts) and a final supernatant solution that contained soluble cell material, microsomes, and some chloroplast fragments. Pellets were homogenized in known volumes of water. Aliquots of the fractions were used for protein and chlorophyll determinations. The lipids were extracted according to Bligh and Dyer (15).

For the nonaqueous isolation of the chloroplasts (3), small pieces of leaves were frozen in liquid nitrogen. Frozen leaves were dried at −20°C under vacuum. 2 g of dried leaves was ground in a Virtis homogenizer with small glass beads in ice-cold hexane–CCl_4 30:70, d 1.32, for 1 min at medium speed. The homogenate was filtered through four layers of cheesecloth. The material that stayed in the cheesecloth was rehomogenized in a Ten Broeck type of homogenizer and added back to the first filtrate. A few unbroken pieces of tissue were removed. A portion of this homogenate was saved. The remaining homogenate was centrifuged at 15,000 *g* for 30 min. The supernatant solution which contained the floating chloroplasts and the chloroplasts that were stuck to the side wall of the tube was transferred into another tube; the pellet, which will be referred to as the "heavy pellet," was saved. About an equal volume of hexane was added to the supernatant solution and the mixture was centrifuged at 3000 *g* for 10 min to yield a pure chloroplast pellet and a clear, light green solution.

Aliquots of the homogenate were saved for chlorophyll and protein determination and the rest was evaporated to dryness under a stream of nitrogen at room temperature. The heavy pellet and the chloroplast pellet were homogenized in water; aliquots were used for chlorophyll and protein determination, and the remaining portions were used for lipid analysis. The light green solution was evaporated to dryness under a stream of N_2 after aliquots had been removed for chlorophyll and protein determination, and the residue was dissolved in chloroform for lipid analysis. The lipids of the other fractions were extracted as before.

Chlorophyll was determined according to Arnon (16) and protein by the method of Lowry, Rosebrough, Farr, and Randall (17).

Lipids were separated by two-dimensional thin-layer chromatography on Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.). The plates were developed in chloroform–methanol–7 N NH_4OH 65:30:4 in the first direction and in chloroform–methanol–acetic acid–water 170:25:25:6 in the second direction (18).

The developed plates were dried in air for 20 min and sprayed with 0.2% 2′,7′-dichlorofluorescein in 95% ethanol. Lipid spots were viewed under UV light and marked.

Various radioactive lipid compounds were prepared enzymatically and their locations on the thin-layer chromatograms were ascertained by radioautography. Monogalactosyl and digalactosyl diglycerides were labeled in their galactose moieties by incubation of spinach chloroplasts with UDP-galactose-^{14}C. Radioactive sulfolipid and phospholipids were prepared by Mr. S. Sumida of this laboratory by incubation of *Chlorella* cells with $^{35}SO_4$ and $^{32}PO_4$, respectively.

There was only one ninhydrin-positive phospholipid on the thin-layer chromatograms. Water-soluble products of acid hydrolysis of this compound gave only one ninhydrin-positive spot which corresponded to ethanolamine in three different solvent systems. The identification of this compound as ethanolamine was also verified with a Beckman 120 B amino acid analyzer. Identification of phosphatidyl choline was substantiated by cochromatographing it with commercially obtained egg lecithin (product of Nutritional Biochemicals Corporation, Cleveland, Ohio). Phosphatidyl inositol and phosphatidyl glycerol were further identified by cochromatography of their water-soluble acid hydrolysis products with inositol, glycerol, and glycerol phosphate.

The R_f values for different lipids in the first and second developing solvents, respectively, were 0.81 and 0.81 for monogalactosyl diglyceride, 0.42 and 0.37 for digalactosyl diglyceride, 0.44 and 0.26 for sulfolipid, 0.31 and 0.51 for phosphatidyl choline, 0.44 and 0.64 for phosphatidyl ethanolamine, 0.55 and 0.49 for phosphatidyl glycerol, 0.15 and 0.16 for phosphatidyl inositol, and 0.16 and 0.78 for phosphatidic acid.

For quantitative analysis of the lipids, the spots were collected from four plates and the lipids were extracted once with 4 ml of chloroform–methanol 1:1, twice with chloroform–methanol 1:2, and once with methanol.

Glycolipid extracts were evaporated to dryness under vacuum and the residue was dissolved in 3 ml of methanol. 3 ml of 2 N HCl was added to each sample and the lipids were hydrolyzed at 100°C for 45 min in open flasks. Fatty acids and dichlorofluorescein were removed with ether and the aqueous phase was evaporated to dryness under vacuum.

The residue was dissolved in 2 ml of water and the amount of sugar was determined according to Dubois, Gilles, Hamilton, Rebers, and Smith (19), with galactose as the standard.

Phospholipid extracts were evaporated to dryness in digestion flasks under a stream of N_2, and the amount of P in each compound was determined by the method of Fiske and Subbarow (20).

RESULTS

Fractionation in Sucrose Solution

The distribution of protein and chlorophyll in the subcellular fractions isolated after centrifugation in 0.5 M sucrose is shown in Table 1. The protein to chlorophyll ratio in the homogenate was 21 whereas that in the chloroplast-rich fraction was, as expected, much lower (5.2). The ratio for the high-speed supernate was 280, the major part of the protein being found in this fraction. The chlorophyll content of the 15,000 *g* pellet is a reflection of the amount of broken chloroplasts in the fraction. The chlorophyll content of the fractions was used to correct values of lipid distribution for contamination by chloroplast material.

Table 2 shows the distribution of glycerolipids after correction for contamination by chloroplast material. The lipids were analyzed on the basis of either phosphate or carbohydrate obtained after hydrolysis of the isolated lipid. The amount of lipid was calculated from the phosphate or sugar determination by using a molecular weight for the various compounds that was derived from an assumed mean fatty acid chain length of 18. We also assumed that the phosphatidyl inositol contains only one phosphate residue. The results show that the chloroplast fraction is particularly rich in the galactosyl diglycerides, sulfolipid, and phosphatidyl glycerol. The largest content of phosphatidyl choline and phosphatidyl ethanolamine was found in the 15,000 *g* supernate and may be due to the presence of microsomes. Phosphatidyl inositol was found mainly in the 15,000 *g* pellet.

The ratios of monogalactosyl diglyceride to other lipids are shown in Table 3. The ratios for digalactosyl diglyceride, sulfolipid, and phosphatidyl glycerol are approximately the same in the chloroplast fraction and in the unfractionated filtrate, which is to be expected since these lipids are concentrated in the chloroplast. The ratios for phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol are much larger in the chloroplast fraction than in the unfractionated filtrate, as would be expected if these lipids are associated with subcellular fractions other than the chloroplast.

Fractionation in Hexane–Carbon Tetrachloride

The distribution of protein and chlorophyll in fractions of tobacco leaves separated by the nonaqueous method in hexane–carbon tetrachloride is shown in Table 4. The major part of both protein and chlorophyll is found in the "heavy pellet" which contains nuclei, mitochondria, microsomes, water-soluble protein, and chloroplasts. The isolation of a pure chloroplast fraction by this technique requires that some chloroplasts be discarded in the heavy pellet. The chloroplasts of the chloroplast fraction were examined microscopically and found to be intact and pure. The protein to chlorophyll ratio decreased, as expected, from 10.9 in the homogenate to 6.4 in the chloroplast fraction. The protein:chlorophyll ratio in the chloroplast fraction is close to that (5.2) for chloroplasts

TABLE 1 DISTRIBUTION OF PROTEIN AND CHLOROPHYLL IN SUBCELLULAR FRACTIONS OF TOBACCO LEAF SEPARATED BY THE AQUEOUS TECHNIQUE

	Filtrate	Pellets						Supernate		Recovery	
		200 *g*		1000 *g*		15,000 *g*					
	mg	*mg*	%	*mg*	%	*mg*	%	*mg*	%	*mg*	%
Protein	1430	34.7	2.5	157	11.5	248	18	932	68	1371.7	96
Chlorophyll	68.7	2.3	3.5	30.2	44	32.8	47.5	3.4	5	68.7	100

The 1000 *g* pellet is the chloroplast-rich fraction.

TABLE 2 DISTRIBUTION OF GLYCEROLIPIDS IN THE FRACTIONS SEPARATED BY THE AQUEOUS TECHNIQUE

	Filtrate	Pellets						Supernate		Recovery	
		200 *g*		1000 *g*		15,000 *g*					
	mg	*mg*	%	*mg*	%	*mg*	%	*mg*	%	*mg*	%
MG	49.3	0	0	35.7	83	3.6	8	4.0	9	43.3	88
DG	31.7	0	0	26.0	88	0.8	3	2.8	9	29.6	93
SL	12.8	0.1	1	8.5	76	1.6	14	1.0	9	11.2	87
PG	12.0	0.15	1	8.5	74	1.4	13	1.4	12	11.4	95
PC	13.3	0.2	1.5	3.4	27	1.6	12.5	7.5	59	12.7	96
PE	6.0	0.05	1	2.3	38	1.2	19	2.6	42	6.1	102
PI	4.2	0.05	1	0.55	13	2.4	56	1.3	30	4.3	102

Percentages in the fractions are calculated on the basis of recovered lipid. Percentage recovery is based on the lipid present in the unfractionated filtrate. Data presented were obtained from original data by correction for chloroplast contamination of each fraction on the basis of chlorophyll content.

Abbreviations: MG, monogalactosyl diglyceride; DG, digalactosyl diglyceride; SL, sulfoquinovosyl diglyceride; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PA, phosphatidic acid.

TABLE 3 RATIOS OF MONOGALACTOSYL DIGLYCERIDE TO OTHER LIPIDS IN FRACTIONS SEPARATED BY THE AQUEOUS TECHNIQUE

	MG	DG	SL	PG	PC	PE	PI
Filtrate	1.0	1.55	3.85	4.1	3.7	8.2	11.7
1000 *g* pellet	1.0	1.4	4.2	4.2	10.5	15.5	65.0

Calculated from the data in Table 2 by dividing weight (mg) of MG by weight (mg) of each other lipid.
See Table 2 for abbreviations.

TABLE 4 DISTRIBUTION OF PROTEIN AND CHLOROPHYLL IN FRACTIONS SEPARATED BY THE NONAQUEOUS TECHNIQUE

	Homogenate	Heavy Pellet		Chloroplasts		Solvent		Recovery	
	mg	*mg*	%	*mg*	%	*mg*	%	*mg*	%
Protein	625	535	91	51.7	8.8	1.4	0.2	588	94
Chlorophyll	57.4	47.9	82	8.1	14	2.5	4	58.5	102

isolated from the aqueous medium. Only 0.2% of the protein was found in the supernatant fraction after the chloroplasts had been centrifuged down, and 4% of the chlorophyll was found in this fraction.

The lipids of the fractions were separated by two-dimensional thin-layer chromatography. Plates charred with sulfuric acid–dichromate are shown in Fig. 3. (Figs. 1 and 2 will be referred to later.) The lipids of the heavy pellet show two spots in the area of phosphatidic acid. Since these are not present in the lipids of the unfractionated filtrate, they are probably an artefact of the preparation of the sample.

The analysis of glycerolipid distribution is shown in Table 5. No attempt has been made to correct for chloroplast contamination of the heavy pellet since lipid analyses indicate that the chlorophyll distribution is not a suitable criterion for the distribution of complex lipids. This is due to the differential extraction of various lipids by the solvent. The most striking feature of the analysis is that the supernatant fraction (the hexane–CCl_4 solution), which contains neither subcellular organelles nor free protein, contains 4% of the chlorophyll (Table 4) and substantial amounts of all other lipids. Among the glycolipids the monogalactosyl diglyceride appears to be particularly easily dissolved by this solvent; among the phospholipids, the phosphatidyl choline appears to be the most readily dissolved. On a percentage basis, all of the glycerolipids are more readily dissolved in the hexane–carbon tetrachloride than is chlorophyll.

The ratios of monogalactosyl diglyceride to the other lipids in chloroplasts obtained by this method are shown in Table 6. The ratios are in good agreement with those calculated for fractionation in the aqueous medium (Table 3), and indicate that isolation by the nonaqueous technique does not drastically change the composition of glycerolipids of chloroplasts. In other experiments where the homogenate was filtered and the residue not rehomogenized, as much as 65% of the monogalactosyl diglyceride was found in the organic solvent. During filtration all the solvent passes through the filter but a good percentage of the particulate material sticks to the filter. The resulting filtrate contains extracted lipids not only from the subcellular material in the filtrate but also from those particles that stick to the filter. Thus it was necessary to rehomogenize the residue and combine it with the filtrate to eliminate this source of error.

Enzymic Activities of the Preparations

The chloroplasts isolated by the nonaqueous technique have been tested for galactolipid synthesis from UDP-galactose-^{14}C. The chloroplasts isolated by the nonaqueous technique show detectable though low enzymatic activity.[1] It has not yet been determined whether activity is limited because of extraction of galactose acceptor, e.g., diglyceride, during the preparative procedure. These chloroplasts were unable to synthesize long-chain fatty acids from acetate-^{14}C, although nonvolatile water-soluble compounds were formed.[1] The formation of phosphatidic acid in the fractions isolated by the nonaqueous technique shows that phospholipases are active.

Electron Microscopy

After potassium permanganate fixation the grana of chloroplasts isolated by aqueous and nonaqueous techniques appeared virtually identical. The partition complex of the grana in both preparations was resolved into two electron-opaque lines separated by an electron-transparent region. This electron-transparent region was

[1] Ongun, A., K. A. Devor, and J. B. Mudd. Unpublished experiments.

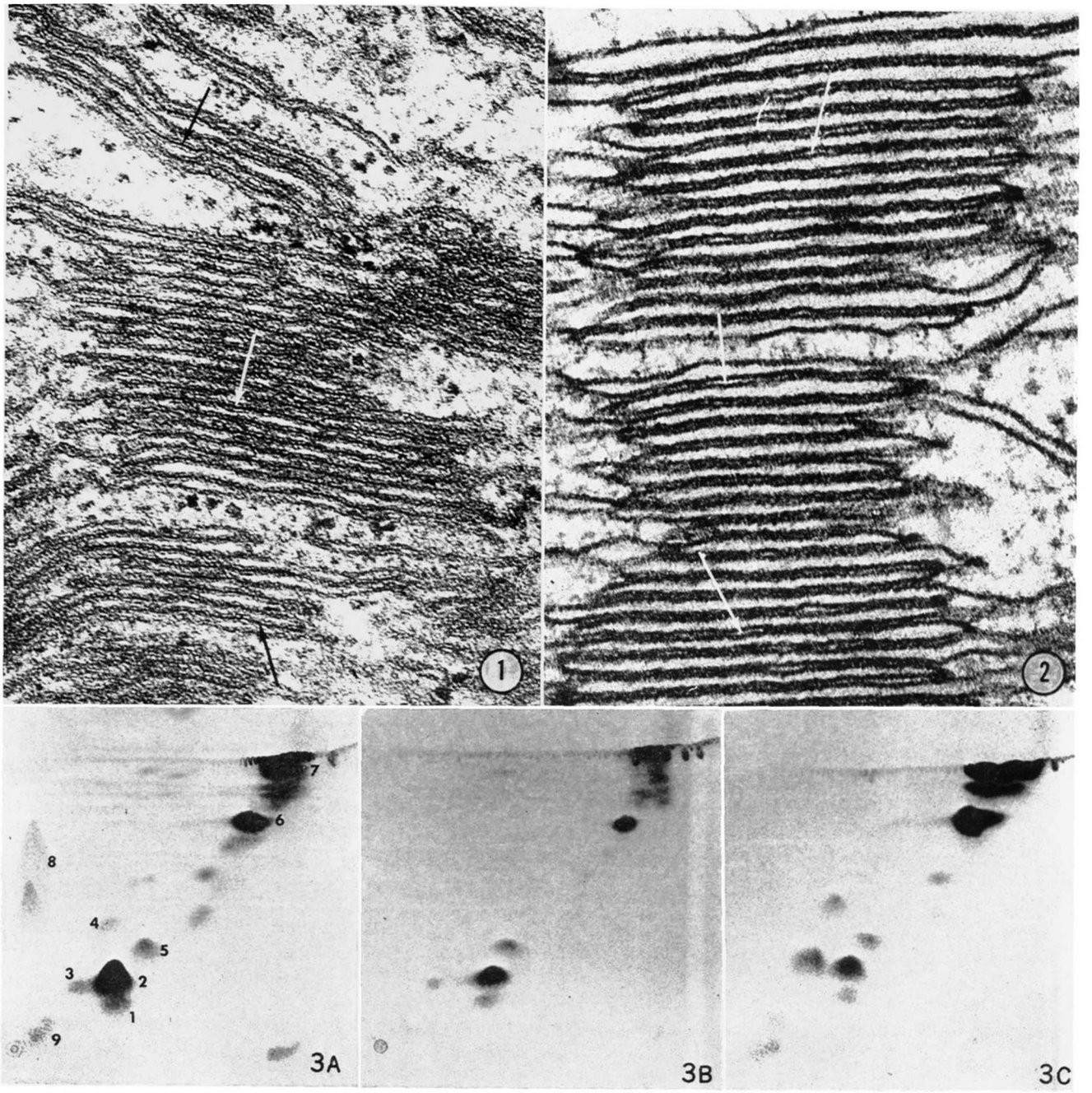

FIG. 1. Grana from a chloroplast isolated by the nonaqueous technique, fixed in osmium tetroxide, and dehydrated at low temperatures. The arrows indicate the dark layer in the center of the partition. ×132,000.

FIG. 2. Grana of a chloroplast fixed with osmium tetroxide in situ and dehydrated at low temperatures. The arrows indicate the electron-transparent region in the center of the partitions. ×162,000.

FIG. 3. Two-dimensional thin-layer chromatography of lipids in cellular fractions separated by the nonaqueous technique. A, heavy pellet; B, chloroplasts; and C, supernatant solution. The origin is at the bottom left of the plates. The first developing solvent moved from left to right and the second from bottom to top. Identification of compounds: *1*, sulfoquinovosyl diglyceride; *2*, digalactosyl diglyceride; *3*, phosphatidyl chlorine; *4*, phosphatidyl ethanolamine; *5*, phosphatidyl glycerol; *6*, monogalactosyl diglyceride; *7*, pigments and neutral lipids; *8*, phosphatidic acid; *9*, phosphatidyl inositol.

TABLE 5 DISTRIBUTION OF LIPIDS IN THE FRACTIONS SEPARATED BY THE NONAQUEOUS TECHNIQUE

	Homogenate	Heavy Pellet		Chloroplasts		Solvent		Recovery	
	mg	*mg*	%	*mg*	%	*mg*	%	*mg*	%
MG	57.9	37.4	67	5.4	10	13.0	23	55.8	97
DG	35.9	28.5	83	3.85	11	2.1	6	34.4	96
SL	14.4	10.4	83	1.35	11	0.8	6	12.5	87
PG	12.9	7.1	72	0.9	9	1.9	19	9.9	77
PC	10.6	5.5	68	0.5	6	2.1	26	8.1	76
PE	5.1	3.25	77	0.2	4.5	0.8	18.5	4.2	82
PI	3.3	2.8	88	0.2	5	0.2	7	3.2	97
PA	0	1.8	—	0.3	—	0	—	2.1	—

Percentages in the fractions are based on the recovered lipid. Percentage recovery is based on the lipid in the unfractionated homogenate.
See Table 2 for abbreviations.

TABLE 6 RATIOS OF MONOGALACTOSYL DIGLYCERIDE TO OTHER LIPIDS IN FRACTIONS SEPARATED BY THE NONAQUEOUS TECHNIQUE

	MG	DG	SL	PG	PC	PE	PI
Homogenate	1.0	1.6	4.0	4.5	5.45	11.3	17.6
Chloroplasts	1.0	1.4	4.0	5.9	11.5	28.5	32.0

Calculated from the data in Table 5 by dividing weight (mg) of MG by weight of each other lipid.
See Table 2 for abbreviations.

also present (Fig. 2, arrows) in chloroplasts fixed by osmium tetroxide in situ and dehydrated at low temperature. However, with osmium tetroxide fixation and dehydration at −20°C the partition complex of the chloroplasts isolated by the nonaqueous technique was strikingly different. The membranes were less smooth and an electron-opaque line was present along the middle of the partition (Fig. 1, arrows). In chloroplasts isolated by the aqueous method the partition appeared after osmium tetroxide fixation as a single dense band (21).

The partitions of the nonaqueously isolated and osmium tetroxide-fixed chloroplasts measured about 140 A and the single membranes at the intragranal connection, the "frets," measured about 73 A. After nonaqueous isolation and permanganate fixation the partitions measured 155 A and the "fret" membranes, 85 A. In osmium tetroxide-fixed, aqueously isolated chloroplasts and in in situ preparations the partitions measured 135–140 A and the "frets" about 75 A.

In summary, there is little difference in size and morphological appearance between grana of aqueously and nonaqueously isolated chloroplasts except for the occurrence of an electron-opaque band in the center of the partitions of the osmium-fixed, nonaqueously isolated chloroplasts. This band or layer has been noted previously in chloroplasts of algae and higher plants but as yet no clear explanation of its presence or absence in different preparations has been offered.

DISCUSSION

Some reports of the distribution of lipids in chloroplast fractions of photosynthetic tissue have been made previously. Wintermans' analyses (22, 23) were made after separation of the deacylated lipids, in contrast to our separation of intact lipids. The lipid fractions he measured were of the whole leaf and of the isolated chloroplasts. The lipids of the cytoplasmic fraction were calculated by difference. Our analyses were made on a larger number of subcellular fractions than those of Wintermans, but the analyses are in agreement on the major points: that the glycolipids and phosphatidyl glycerol are concentrated in the chloroplast and that phosphatidyl choline is mainly in the cytoplasmic fraction. A separate analysis of the lipids of chloroplast lamellae made by Allen, Good, Davis, Chisum, and Fowler (24) is also in reasonably good agreement with the data we have obtained for the chloroplast fraction.

The analyses of glycerolipid distribution in photosynthetic tissue fractionated by nonaqueous techniques, which have not been reported before, may now be compared with lipid analyses of fractions isolated in aqueous media. Our data clearly show that as much as 25% of the lipid in the tissue is extracted in the hexane–carbon tetrachloride solvent. It is clear from microscopic examination that chloroplasts isolated by the nonaqueous technique do not have disrupted chloroplast structure. Extraction of chloroplasts by heptane and petroleum

ether removed more than 90% of the plastoquinones but the grana structure was retained, although there was a pronounced decrease in the width of the membranes (25). In the accompanying paper (21) we have shown that vigorous extraction of lipids from the chloroplast by mixtures of chloroform and methanol prevents the subsequent demonstration of chloroplast structure; however, it is clear from experiments with hexane–carbon tetrachloride that some lipid can be extracted without drastic changes in the membrane structure. In the case of bovine heart mitochondria, Fleischer, Fleischer, and Stoeckenius (26) have reported that 95% of the lipid can be extracted while the unit membrane structure is retained.

In the experiment reported in detail in this paper, the glycerolipid composition of chloroplasts isolated by the nonaqueous technique was similar to that of chloroplasts isolated in aqueous media. However, the organic solvents tend to extract monogalactosyl diglyceride, and probably other lipids that were not assayed in this study, such as plastoquinones. This view is supported by the lipid distribution shown in Fig. 3. A further problem in the use of nonaqueously isolated chloroplasts for enzymic studies is the activation of phospholipases as encountered in this study. It is apparently necessary to remove organic solvent completely from the isolated pellets before these are resuspended in aqueous media, if the activation of the phospholipases is to be prevented.

One of the advantages of the isolation of chloroplasts in organic media is the retention of stroma protein. There is undoubtedly some variation in the loss of protein depending on the species of plant used. In the case of *Oenothera* sp. (evening primrose) it has been found that after fractionation in sucrose–phosphate 75% of the leaf protein is in the plastids (27). This figure is as high as that usually found with the nonaqueous technique. An average figure for plastid protein as percentage of total protein when fractionation is in aqueous media is 35%. The data presented in this paper may be used to calculate chloroplast protein as percentage of the whole protein. For the nonaqueous isolation it is 63%, whereas it is 26% for the aqueous isolation. However, the protein: chlorophyll ratio is not so different in the two chloroplast preparations: 6.4 for the nonaqueously isolated chloroplasts, and 5.2 for those isolated in the aqueous medium. The protein:chlorophyll ratio is quite different in the unfractionated preparations. It is the relatively high amount of protein in the unfractionated aqueous homogenate which makes the recovery of protein in the chloroplast apparently low, even though the protein: chlorophyll ratio approaches that found in the nonaqueously isolated chloroplasts.

Electron microscopic examination of the chloroplasts isolated in nonaqueous media shows a marked difference from aqueously isolated or in situ preparations when the fixation is by osmium tetroxide (Figs. 1 and 2). There was relatively little difference when the fixation was by potassium permanganate. In neither osmium tetroxide nor potassium permanganate fixation was there any marked change in the dimensions of the membranous structures of the chloroplast.

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