Lipid composition of plant mitochondria and of chloroplasts

Harvey A. Schwertner[1] and Jacob B. Biale

Biology Department, University of California, Los Angeles, California 90024

Abstract The mitochondrial lipids from avocado fruit, cauliflower buds, and potato tubers, and the lipids of chloroplasts isolated from avocado fruit and from cauliflower leaves were identified and the concentrations were determined. The lipid composition was compared with that of beef heart mitochondria. Phospholipids constituted 50–56% of total lipids in plant mitochondria while this fraction made up 90% of the lipids in beef heart mitochondria. In both cases the chief phospholipids were phosphatidylcholine and phosphatidylethanolamine. A characteristic feature of plant mitochondria was the presence of monogalactosyl- and digalactosyldiglyceride and of sulfolipid. Potato mitochondria differed from the particles of other species investigated by their higher content of galactolipids, sterol glycosides, and carotenoids and lower content of phospholipids and of total lipids in the lipid–protein complex.

The galactolipid content was markedly higher in chloroplasts from all sources than in mitochondria. The spectrum of lipids in the phospholipid fraction differed more strikingly between chloroplasts of the leaf and the mitochondria of the bud of cauliflower than between the two organelles of the avocado mesocarp. The fatty acid distribution of individual lipids and of classes of lipids was also more similar in the two organelles of the fruit tissue than in the cauliflower material.

Supplementary key words phospholipids · galactolipids · fatty acids · sterols

When this study was undertaken, only scant information was available on the lipid composition of plant mitochondria. In a recent monograph on plant lipid biochemistry (1), the statement was advanced that though mitochondria play an essential role in plant metabolism "little quantitative data are available regarding their lipid composition." The lipids from plant materials were obtained mostly from tissue rather than from subcellular fractions, as exemplified by the studies on potato tuber (1) and on fruits (1–5). The phospholipid and fatty acid contents of apple mitochondria were found by Mazliak, Ben Abdelkader, and Catesson (6) to be about the same as in the whole tissue. Douce et al. (7) observed differences between mitochondria and chloroplasts with respect to the spectrum of individual phospholipids. The absence of phosphatidylethanolamine and diphosphatidylglycerol in chloroplasts was especially noteworthy. In a preliminary study on isolated avocado mitochondria, Biale, Yang, and Benson (8) reported the presence of monogalactosyl- and digalactosyldiglyceride in addition to the common components of phospholipids. The relatively high galactolipid content was ascribed to contamination by plastids and prompted the execution of analytical studies on purified subcellular fractions by the adaptation of modern chromatographic techniques.

EXPERIMENTAL PROCEDURE

Isolation and purification of organelles

Chloroplasts were prepared by homogenization of the outer green tissue of Hass avocado fruit according to the method of Spencer and Wildman (9), and mitochondria were isolated from the inner yellow mesocarp by the procedure of Hobson et al. (10). Chloroplasts were also obtained from the intact leaves surrounding cauliflower heads, and mitochondria were prepared from the surface 1–2 mm of the white heads, which are the buds of the cauliflower. The age of the material was not known, but it was fully mature and freshly harvested. The isolation medium included 0.4 M sucrose for mitochondria and 0.5

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; DEGS, diethylene glycol succinate; BSA, bovine serum albumin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; MGD, monogalactosyldiglyceride; DGD, digalactosyldiglyceride; SL, sulfolipid.

[1] Present address: Biochemistry Branch, Environmental Systems Division, USAF School of Aerospace Medicine, Brooks AFB, Texas 78235.

M sucrose for chloroplasts. The other components of the medium and the centrifugation sequence were described previously (10). Mitochondria of animal origin were prepared by the method referred to in Fleischer et al. (11) from beef hearts obtained from freshly slaughtered animals.

The mitochondrial pellet obtained from the precipitate of differential centrifugation (10) was purified by centrifugation in continuous density gradients of iothalamic acid (12) prepared by gravity flow of a solution containing 60 mg of iothalamic acid/ml of isolation medium into a mixing chamber containing 200 mg/ml of iothalamic acid. The gradient had a concentration range of approximately 107 mg/ml at the top to 200 mg/ml at the bottom of the 30-ml centrifuge tube. The isolation medium consisted of 0.4 M sucrose, 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid, 10 mM KH_2PO_4, 10 mM KCl, 10 mM EDTA, and 1 mg/ml BSA, pH 7.8. Chloroplasts were purified by a discontinuous gradient made up of 10 ml of sucrose (60%) at the bottom of the tube, 10 ml of sucrose (45%) as intermediate layer, and 5 ml of sucrose (20%) as top layer. The sucrose solutions contained 2 mM phosphate buffer maintained at pH 7.8 to prevent enzymatic hydrolysis of lipids. Both mitochondrial and chloroplast gradients were centrifuged for 1 hr at 14,900 *g* in a Spinco SW-25.1 rotor. The chloroplasts and mitochondria were removed from the centrifugation tubes by a syringe or by gravity flow of a concentrated solution into the bottom of the tube forcing the gradient out of the top. The separated fractions were centrifuged and resuspended in the assay medium for the polarographic determination of oxidative activity, as described by Hobson et al. (10). Protein content of the organelles was determined by the method of Lowry et al. (13), using BSA as standard.

Preparation of lipids

Immediately after purification, the organelles were denatured with boiling 80% ethanol and centrifuged, and the pellet was extracted three times with 5 ml of chloroform–methanol 2:1. The combined ethanol and chloroform–methanol extracts were purified by removal of nonlipid substances according to the procedures of Folch, Lees, and Sloane Stanley (14). Total lipids were estimated by weighing an aliquot of the extract dried under nitrogen. Aliquots were taken also for the analyses of chlorophyll content (15), total lipid phosphorus (16), and total fatty acid by GLC. The lipid solutions were stored under nitrogen in screw-cap culture tubes sealed with Teflon liners.

TLC separations of lipids

Individual lipids or lipid classes were obtained directly by TLC of total lipid aliquots, thereby eliminating the column chromatographic steps which seldom yield pure individual lipids or lipid classes. Acetone or acetone–acetic acid–water 100:2:1 was effective in separating MGD, DGD, and SL (17). The acetone solvents also enabled the separation of sterol glycosides, esterified sterol glycosides, and phytocerebrosides (Table 1). Acetone–acetic acid 100:1 resulted in the separation of the neutral lipids as a class from the galactolipids, and acetone–acetic acid–water 100:2:1 separated the galactolipids and sulfolipid from the phospholipid class. The purified neutral lipids and phospholipids could then be resolved into individual lipids with hexane–ethyl ether–acetic acid 80:20:1 and chloroform–methanol–water 65:25:4 (Table 1). The addition of acetic acid to the acetone solvents is required to prevent trailing, loss of neutral lipid fatty acids, and contamination of the other lipids.

Characterization of lipids

The nature of most polar lipids was verified by standard chemical procedures such as the identification of hydrolysis products (1, 18). The deacylated lipids were compared with products of known phospholipid standards. The galactolipids were identified and estimated after the elution of the spots from the chromatograms (1, 18) and the determination of galactose in the eluate (19). Additional confirmation of individual lipids was achieved by chromatography on TLC plates with lipid standards. The detection reagents included sprays of rhodamine 6G, molybdate, and ninhydrin, and iodine vapor (1, 20). Galactolipids were detected by spraying the plates with 10% phenol–H_2SO_4 1:1 (v/v) followed by heating. Perchloric acid (20%) was useful in tentatively identifying the sterol glycosides (1). The phytocerebrosides were cochromatographed with calf brain cerebrosides using acetone solvents referred to above (17) for the development of the TLC plates. Quinones were identified by the method of Barr, Henninger, and Crane (21).

Quantitative analysis of individual lipids

Phospholipids were separated on TLC, and after elution of each identified spot phosphorus was determined by the method of Chen, Toribara, and Warner (16). Galactolipids and sulfolipids were estimated quantitatively (1). Fatty acid methyl esters were prepared for GLC analysis by transesterification of the lipids with anhydrous 5% HCl–methanol or 14% BF_3–methanol (1, 22). Erucic acid was used routinely as an internal standard. After transesterification, the methyl esters and the nonsaponifiable lipids were extracted, washed, and separated on silica gel columns (0.5 × 15 cm). The relatively nonpolar carotenes and hydrocarbons were eluted with hexane, and the fatty acid methyl esters with 3% ether in hexane. The fraction was evaporated and dissolved in benzene or hexane for GLC analysis.

The fatty acid composition of the methyl esters was

TABLE 1. R_F values of lipids extracted from plant organelles and separated on TLC by three solvent systems

Lipid	Solvent A	Lipid	Solvent B	Lipid	Solvent C
Pigments and neutral lipids	0.99	Pigments and neutral lipids	0.90	Quinones	0.90
Sterol glycoside fatty acid esters	0.93	Sterol glycoside fatty acid esters	0.79	Carotenes and sterol fatty acid esters	0.85
Monogalactosyldiglyceride and diphosphatidylglycerol	0.83	Monogalactosyldiglyceride	0.67	Fatty acid methyl esters	0.69
		Sterol glycosides and phytocerebrosides	0.45	Triglycerides	0.66
				Free fatty acids	0.42
Sterol glycosides	0.70	Digalactosyldiglyceride	0.26	Quinones	0.28
Phosphatidic acid and phytocerebrosides	0.66	Sulfolipid	0.11	Free sterols and diglycerides	0.15
		Origin, phospholipids			
Digalactosyldiglyceride and phosphatidylethanolamine	0.55			Monoglycerides, chlorophylls and xanthophylls	0.07
				Origin, polar lipids	
Phosphatidylglycerol	0.36				
Phosphatidylcholine and sulfolipid	0.30				
Phosphatidylinositol	0.23				
Phosphatidylglycerine	0.09				
Origin					

Solvent A, chloroform–methanol–water 65:25:4; solvent B, acetone–acetic acid–water 100:2:1; solvent C, hexane–diethyl ether–acetic acid 80:20:1. Phosphatidic acid and phytocerebroside have not been conclusively identified. Phosphatidic acid gave a positive reaction with molybdenum blue spray (1), and phytocerebrosides had chromatographic properties similar to calf brain cerebrosides.

determined by GLC on an F & M model 810 gas chromatograph equipped with dual columns (6 ft × 1/8 inch O.D.) containing 10% DEGS on 80–100 mesh Diatoport S. With these columns, hexadecatrienoic acid, 16:3, and oleic acid, 18:1, peaks overlapped. The amounts of these acids were determined by calculating the changes in palmitic acid, 16:0, and stearic acid, 18:0, before and after hydrogenation with $PtCl_2$. Subsequent analyses with columns containing 15% DEGS resulted in complete separation of 16:3 and 18:1 acids without interfering with the other fatty acids.

RESULTS

Mitochondria isolated by differential centrifugation were further purified by continuous density gradient centrifugation. Iothalamic acid gradients appeared to give slightly better separation and a more concentrated band of particles than did gradients of sucrose or of rhenographin. Cauliflower, avocado, and beef heart mitochondria separated in the region of the gradient corresponding approximately to a concentration of 130 mg/ml of iothalamic acid; mitochondria from Irish potato appeared at a slightly greater density. Succinoxidase activity was associated with the well-defined band of mitochondrial particles. In the case of avocado mesocarp, the rate of succinate oxidation increased from approximately 900 to 1300 μl of O_2/mg of protein nitrogen/hr as a result of purification. The purity of mitochondrial preparations was monitored by phase-contrast microscopy. Relatively homogeneous preparations of mitochondria could be obtained without density gradient purification from potato tuber and from cauliflower buds, whereas mitochondria obtained from avocado fruit by differential centrifugation contained some chloroplast fragments and 2% more lipid than did the density gradient-purified particles. Beef heart mitochondria obtained by the regular procedure could be further purified by gradient centrifugation as judged by the removal of heme pigments and lighter material.

Mitochondria could not be isolated from green leaves without some chlorophyll contamination, even by the density gradient techniques. The yields of cytoplasmic particles from such tissue as etiolated peanut cotyledons or barley roots were too low for extensive lipid analyses. The relative ease of isolating pure mitochondria from avocado fruit, cauliflower buds, and potato tuber in contrast to leaf or fibrous root material may, in part, be due to differences in mitochondrial content of the tissue. The bulk of cauliflower chloroplasts appeared in the bottom of the 45% sucrose layer while avocado chloroplasts concentrated in the bottom of the 20% layer.

Lipid composition of cauliflower and avocado mitochondria and chloroplasts

The composition of individual classes of lipids as well as the total lipid content are presented in Table 2. The percentage of total lipid was similar for both organelles in avocado and in cauliflower. The phospholipid content in relation to total lipids was considerably higher in mitochondria than in chloroplasts. Avocado chloroplasts contained much less MGD and chlorophyll and more phospholipid than cauliflower chloroplasts; however, the DGD and SL contents were about the same in the two species.

TABLE 2. Lipid composition of avocado and cauliflower mitochondria and chloroplasts

	Avodado		Cauliflower	
	Mitochondria	Chloroplasts	Mitochondria	Chloroplasts
Total lipid,[a] %	38	38	33	34
	weight % of total lipid[b]			
Phospholipid	50.0	20.4	54.0	9.1
Monogalactosyldiglyceride	2.1	9.0	2.2	19.4
Digalactosyldiglyceride	3.0	6.7	2.7	7.4
Sulfolipid	0.9	3.6	1.3	2.5
Chlorophyll	0.04	8.2	0.03	33.6
Fatty acids of total neutral lipid	16.5	ND	18.9	18.2
	nmoles/mg protein N			
Phospholipid	2455	995	2467	365
Monogalactosyldiglyceride	101	360	99	777
Digalactosyldiglyceride	122	223	102	248
Sulfolipid	39	135	56	95
Chlorophyll	2	286	2	1172
Fatty acids of total neutral lipid	2394	ND	2265	1922

ND, not determined.

[a] Total lipid = (lipid/lipid + protein) × 100.

[b] Nanomoles of lipid were converted to weight using the following calculated molecular weights: monogalactosyldiglyceride, 786.6; digalactosyldiglyceride, 948.6; sulfolipid, 837.0; phospholipid, 784.3; chlorophyll, 905.0.

In mitochondria no differences were found in individual polar lipids between avocado fruit and cauliflower bud. Significantly, plant mitochondria containing virtually no chlorophyll do contain appreciable amounts of galactolipids. The chlorophyll fraction accounted for one-third of total lipids in density gradient preparations of chloroplasts whereas it was closer to one-fifth in nonpurified chloroplasts. The chlorophyll levels varied much more than the galactolipid or sulfolipid content. The traces of chlorophyll calculated for plant mitochondrial lipids may be much lower than those given because chlorophyll determinations were made on total lipid aliquots and lipids other than chlorophyll may be contributing to the absorptivity. A base-line correction beyond 710 nm, where chlorophyll does not absorb light, was not taken but would have corrected for absorption due to other lipids. Avocado and cauliflower mitochondria as well as cauliflower chloroplasts contained appreciable amounts of fatty acids derived from neutral lipids, indicating the presence of a substantial neutral lipid fraction.

Differences in individual phospholipids of mitochondria and chloroplasts are given in Table 3. The most apparent differences are between cauliflower mitochondria and chloroplasts. PE and PC are the primary phospholipids in mitochondria, PG in cauliflower chloroplasts, and PC and PE in avocado chloroplasts.

Sterol glycosides, esterified sterol glycosides, and phytocerebrosides were found in organelles in amounts which probably constitute less than 1% of the total lipids. Undoubtedly, there are other polar lipids present in small amounts which were not identified. No quantitative analyses were made on lipids present in the tissues from which the organelles were isolated. The lipid compositions of potato tubers (1) and of avocado fruit (3, 4, 5) have been thoroughly studied. Esterified and free sterol glycosides were observed to be present in substantial amounts in the cauliflower buds, whereas monogalactosyl- and digalactosyldiglycerides were only minor components.

With the exception of one minor lipid, avocado and cauliflower mitochondria were found to contain the same types and proportions of individual neutral lipids as judged after charring or spraying with the various lipid reagents. Two quinone-positive spots were detected by TLC in both chloroplasts and mitochondria; however, chloroplasts appeared to contain larger quantities. The quinone spot at the top of the plate (Table 1) was the most intense when reacted with reduced methylene blue. In general, spots corresponding to triglycerides and quinones were of equal intensity and more pronounced than spots corresponding to free fatty acids, sterol fatty acid esters, and sterols for both avocado and cauliflower mitochondria. Triglycerides were observed to be present in lower amounts in cauliflower chloroplasts than in mitochondria; avocado chloroplasts and mitochondria appeared to contain equal amounts. The triglycerides and other neutral lipids were much more pronounced in mitochondria of plant material than in mitochondria of beef heart. Further characterization of plant mitochondrial neutral lipids was accomplished by direct gas–liquid chromatographic analysis of the total neutral lipids and will be subsequently reported.

Fatty acid composition of lipids and lipid classes from plant organelles (Table 4)

The fatty acid compositions of individual lipids and of lipid classes were determined on mitochondria isolated from cauliflower buds and on chloroplasts obtained from cauliflower leaves. In general, the distribution of fatty acids of the same lipid or lipid class from cauliflower chloroplasts resembled those of the mitochondria. MGD from chloroplasts contained more linolenic and hexadecatrienoic acids and less short-chain fatty acids than MGD from mitochondria. DGD from chloroplasts also contained more linolenic acid and less short-chain fatty acids than DGD from mitochondria. The fatty acids of the neutral lipids from the two organelles were similar and contained large amounts of short-chain fatty acids and

TABLE 3. Phospholipid spectra of avocado and cauliflower mitochondria and chloroplasts

	Avocado		Cauliflower	
	Mitochondria	Chloroplasts	Mitochondria	Chloroplasts
	mole %			
Phosphatidylcholine	45	34	33	19
Phosphatidylethanolamine	21	33	39	13
Phosphatidylinositol	11	12	10	12
Phosphatidylserine	4	6	5	9
Phosphatidylglycerol	5	6	4	44
Diphosphatidylglycerol	9	9	6	3
Phosphatidic acid	5	0	3	0

palmitic acid. The fatty acid patterns of the total phospholipid class in the two organelles differed, as would be expected, on the basis of their relative amounts and types of phospholipids. The short-chain fatty acids were not identified, but based on retention times, they were thought to consist primarily of C_{14} and secondarily of C_{10}, C_{12}, and C_{13} fatty acids. The presence of unsaturation was indicated upon hydrogenation.

The fatty acid compositions of total lipids and galactolipids were determined on avocado mitochondria and chloroplasts isolated from the same fruit; these data are in the bottom section of Table 4. The chloroplasts were prepared from the outer green tissue and mitochondria from the inner yellow tissue. The fatty acid compositions of the galactolipids were generally the same in the two organelles, though slightly more linolenic acid and less short-chain fatty acids were present in the galactolipids of the chloroplasts than in the mitochondrial fraction. There was little difference in the fatty acid distribution of the total lipids between the two organelles with the exception of linolenic acid. Hexadecatrienoic acid was essentially absent from the galactolipids of avocado chloroplasts.

Fatty acid composition from individual lipids from cauliflower mitochondria and chloroplasts (Table 5)

The fatty acids of the individual phospholipids were determined from cauliflower mitochondria isolated roughly a year later than were the mitochondria and chloroplasts used for the other lipid analyses. This accounts for the difference in the amounts of short-chain fatty acids of the individual phospholipids and those of the total phospholipids (Table 4). Phosphatidylethanolamine and phosphatidylcholine had similar fatty acids but differed from diphosphatidylglycerol, which was characterized by a high linolenic acid content. The fatty acids of chloroplast phosphatidylglycerol and phosphatidylcholine resembled the fatty acids of the total phospholipids (Table 4) as would be expected since phos-

TABLE 4. Fatty acid composition of lipid classes of avocado and cauliflower mitochondria and chloroplasts

	<16:0[a]	16:0	16:1	16:2	16:3	17:0	18:0	18:1	18:2	18:3
					mole %					
Cauliflower										
Total lipids										
Mitochondria	1.6	14.7	0.7	0.1	1.0	0.1	1.3	8.2	14.2	58.0
Chloroplasts	10.7	11.3	4.0	1.5	13.9	—[b]	1.7	9.6	7.2	40.2
Monogalactosyldiglyceride										
Mitochondria	23.9	10.9	2.7	1.5	5.0	4.1	3.3	7.5	5.1	36.1
Chloroplasts	4.8	2.8	0.9	2.8	28.6	0.8	1.4	6.9	3.6	47.5
Digalactosyldiglyceride										
Mitochondria	13.3	11.4	3.0	1.2	2.6	1.7	3.9	7.0	5.6	50.3
Chloroplasts	4.9	8.8	1.8	1.2	0.4	0.8	2.6	5.9	5.7	67.9
Total phospholipids										
Mitochondria	16.4	24.4	1.0	—	—	—	1.0	6.5	17.4	33.4
Chloroplasts	34.6	32.4	16.4	1.0	—	2.0	0.9	1.3	6.4	4.8
Total neutral lipids										
Mitochondria	33.7	25.8	9.4	0.2	—	2.2	0.5	5.8	5.8	16.7
Chloroplasts	42.7	26.9	7.0	0.6	4.4	3.7	0.6	8.7	3.2	2.5
Avocado										
Total lipids										
Mitochondria	0.9	11.4	2.3	0.1	0.4	—	0.6	60.4	16.3	7.6
Chloroplasts	2.8	11.2	1.4	0.1	0.2	—	0.5	57.4	13.2	13.1
Monogalactosyldiglyceride										
Mitochondria	14.1	7.2	3.3	2.4	—	2.5	2.2	35.4	10.8	22.3
Chloroplasts	5.5	4.4	2.1	0.9	0.9	0.9	2.8	43.2	7.8	31.7
Digalactosyldiglyceride										
Mitochondria	11.0	6.7	3.5	1.7	—	1.6	3.4	41.4	9.0	21.8
Chloroplasts	5.5	6.0	1.7	1.3	—	1.1	2.1	46.0	7.8	28.5

[a] Number of carbon atoms:number of double bonds.
[b] Dashes indicate that the acid was not detectable.

TABLE 5. Fatty acid composition of individual lipids from cauliflower mitochondria[a] and chloroplasts

	<16:0	16:0	16:1	16:2	16:3	17:0	18:0	18:1	18:2	18:3
					mole %					
Mitochondria										
Diphosphatidylglycerol	1.4	3.7						3.6	26.2	65.1
Phosphatidylethanolamine	1.4	20.4	1.3				1.2	8.2	28.1	39.4
Phosphatidylcholine		20.5	1.6				1.8	6.4	24.4	45.3
Phosphatidylinositol		44.7	1.1				1.9	3.1	17.4	31.8
Chloroplasts										
Phosphatidylglycerol	28.8	24.1	31.6	1.6		2.0	1.9	2.1	5.2	2.8
Phosphatidylcholine	38.7	30.9	11.0	1.3		9.0	1.2	1.0	4.8	2.1
Sulfolipid	4.1	27.8	16.1				3.2	18.3	11.7	18.8

[a] Mitochondria used for lipid analyses here were obtained at a different time from those used in Table 4.

phatidylglycerol and phosphatidylcholine are among the major phospholipids. Phosphatidylglycerol from actively photosynthesizing tissue is unique in containing both *trans*-3-hexadecenoic acid and *trans*-9-hexadecenoic acid isomers. The 16:1Δ3 of phosphatidylglycerol was not conclusively identified but was assumed to be identical with an unknown fatty acid having a retention time slightly longer than 17:0 and after hydrogenation appearing as palmitic acid. In phosphatidylglycerol, 16:1Δ3 made up 14.8% and 16:1Δ9 made up 16.8% of the total fatty acids. In the total phospholipids, 16:1Δ3 and 16:1Δ9 constituted 6.0 and 10.4% of the fatty acids, respectively. The *trans*-Δ3 isomer was found in only trace amounts in phosphatidylcholine. The fatty acids of the sulfolipids did not resemble those of the galactolipids.

Lipid composition of mitochondria isolated from Irish potato tubers (Table 6)

Mitochondria isolated from Irish potato tubers were found to have a much lower amount of total lipid and phospholipid than either avocado or cauliflower mitochondria, although they contained larger amounts of the galactosyldiglycerides. Sulfolipid was present in potato mitochondria but it was not determined quantitatively. Several lipids were observed to be present in potato mitochondria in significant amounts and resembled esterified sterol glycosides in chromatographic properties but gave several different color reactions with 20% perchloric acid. Carotenoids were detected in the Irish potato mitochondrial lipids by TLC and by spectrophotometric scans of the total lipids. By the same methods, carotenoids were found to be essentially absent as cauliflower and avocado lipid components. Several lipids from potato mitochondria appeared between the carotenes and free fatty acids and gave blue color reactions with iodine or 20% perchloric acid. These spots were not characteristic of lipids from mitochondria of the other species. Of the neutral lipids in potato mitochondria, spots corresponding to free sterols, sterol esters, carotenoids, and several other unidentified lipids appeared to be the principal neutral lipids.

The variations in fatty acid composition between some lipid classes of potato mitochondria resemble those of the avocado organelles. The galactolipids are slightly more unsaturated than are the total lipids, phospholipids, and neutral lipids. The phospholipids, which constitute 56% of the total lipids, resemble the total lipid fatty acids. Oleic acid is the prevalent fatty acid of the neutral lipids.

DISCUSSION

General conclusions regarding lipid compositions of mitochondria and of chloroplasts

The total lipid content of plant mitochondria was found to vary from 38% for avocado to 33% for cauliflower and 17% for Irish potato. Phospholipids constituted 50–56% of the lipid in plant mitochondria. In contrast, mitochondria from rat and mouse liver and from beef and pig heart contain 27–29% lipid, of which 90% is phospholipid (11). Plant mitochondria, therefore, differ from animal mitochondria in containing larger amounts of neutral lipids and lower amounts of phospholipid. Galactolipids and sulfolipid are present in plant mitochondria but are absent from animal mitochondria. Avocado, cauliflower, and beef heart mitochondria appear to have identical densities as determined by iothalamic acid density gradient centrifugations. PC and PE are the predominant phospholipids in both plant and animal mitochondria.

The galactosyldiglycerides and the various sterols were present in much higher amounts in potato than in either avocado or cauliflower mitochondria. Brain mitochondria also contain cerebrosides, sulfatides, and sphingomyelin and high amounts of steroids (23), which appear to distinguish them from liver, kidney, and heart mitochondria (11). Plant mitochondria resemble brain mitochondria with respect to the amounts of phospholipids and sulfur and galactose-containing lipids. However, MGD, DGD, and SL are different chemically from the sulfatides and cerebrosides of brain mitochondria.

TABLE 6. Lipid composition of mitochondria from Irish potato tuber

Total lipid, %	17.6	
	weight % of total lipid	*nmoles/mg protein N*
Phospholipid	56.0	884
Monogalactosyldiglyceride	7.3	114
Digalactosyldiglyceride	15.5	225
Fatty acids of total neutral lipid	14.1	585

Fatty acid composition of mitochondrial lipids from Irish potato tuber

	<16:0	16:0	16:1	18:0	18:1	18:2	18:3
				mole %			
Total lipid	2.0	21.0		5.2	2.1	50.1	19.6
Total neutral lipid	2.8	16.5	3.5	2.1	32.5	26.6	15.9
Total phospholipid		21.0		3.7	1.7	55.0	18.7
Monogalactosyldiglyceride	2.2	14.5	1.0	1.8	4.2	49.2	27.1
Digalactosyldiglyceride		11.9		9.1	2.4	52.8	23.8

The literature on total lipid content of chloroplasts includes results from as low as 21% for whole chloroplasts (24) to as high as 55% for pure lamellar membranes (1, 25). The last value was reduced to 32% when a correction factor (Ref. 24) was applied for possible loss of soluble proteins. In this study, chloroplasts of uniform density were used for lipid analyses. Damaged, swollen, or fragmented chloroplasts were excluded largely by the density gradient procedure. The amounts of galactolipids and phospholipids of cauliflower chloroplasts are in agreement with the glycerolipid compositions of tobacco chloroplasts isolated by both aqueous and nonaqueous techniques (1). Avocado chloroplasts were found to differ appreciably from cauliflower chloroplasts in terms of lipid composition. It is also evident that the phospholipids of cauliflower chloroplasts and of chloroplasts of other species (1, 24) differ distinctly from those of plant mitochondria.

Fatty acid composition of mitochondria and of chloroplasts

The fatty acid composition of plant mitochondria has not been studied extensively and has been limited to the "total lipids" fraction (1, 6). The results on fatty acid content of total lipid of potato and of cauliflower agree with data reported by Lyons, Wheaton, and Pratt (26). The fatty acid specificities of individual phosphatides within plant mitochondria resemble those within animal mitochondria. In both cases, the fatty acid composition of phosphatidylcholine is similar to that of phosphatidylethanolamine and differs from diphosphatidylglycerol and phosphatidylinositol (11). Linoleic acid constitutes 74% and linolenic acid 65% of the fatty acids of diphosphatidylglycerol of animal and of cauliflower mitochondria.

In cauliflower chloroplasts, hexadecatrienoic acid was localized primarily in monogalactosyldiglyceride. As in the case of other tissue (1), hexadecatrienoic acid content correlated with a reduction in the proportion of linolenic acid, resulting in the same total polyenoic acid content of both monogalactosyl- and digalactosyldiglyceride.

Some individual lipids and lipid classes in both cauliflower chloroplasts and mitochondria are very similar in fatty acid patterns. There appears to be even less difference in the fatty acid compositions of individual lipids between the organelles of avocado. Fatty acid compositions of total lipids, phospholipids, and neutral lipids of rat liver mitochondria, nuclei, and microsomes are also very similar (11) as are the fatty acids of mitochondria isolated from apple parenchyma and those of the tissue from which they were isolated (6). Even though the cauliflower organelles were not isolated from the same tissue, they probably reflect more closely organelle differences in fatty acids of individual lipids and in lipid compositions than do the avocado organelles whose chloroplasts, as judged by their densities and lipid compositions, appeared to be either degenerated or not fully developed. At present, the difficulties in isolating pure mitochondria from green leaves is the major limiting factor in the ability to compare lipids of chloroplasts and mitochondria from the same tissue.

Evidence for the occurrence of galactolipids in plant mitochondria

Biale et al. (8) and Schwertner and Biale (27) reported the occurrence of galactolipids in plant mitochondria. Further evidence with highly purified particles from different species indicates that galactolipids on a lipid and on a protein nitrogen basis do account for a significant portion of the lipids. Galactolipids also have been isolated from such nonchlorophyllous tissues as roots of parsnip, pea, and turnip, and potato tubers (1, 28). The lipid compositions of these materials are very similar to the lipid composition of potato tuber mitochondria reported here. It can be assumed that the

source of galactolipids is mitochondria rather than proplastids since chloroplast precursors typical of those found in etiolated leaves are absent from root tissue (1). Carotenoids and phytol, which normally are constituents of proplastids, were not detected in avocado and cauliflower mitochondrial preparations. The isolation procedures used in this study are similar to the one adapted by Baker et al. (29) which yielded pure particles as observed by electron microscopy. Not only are galactolipids present in mitochondrial preparations from pea roots, cauliflower buds, and the inner mesocarp of avocado fruit, but also the enzymes capable of synthesizing these lipids have been isolated (28) from mitochondria of these materials.

This study was supported in part by grants from National Science Foundation, GB-7408; U.S. Public Health Service, GM-08224; and by the University Committee on Research.

Manuscript received 18 November 1971, and in revised form 29 September 1972; accepted 2 November 1972.

REFERENCES

1. Hitchcock, C., and B. W. Nichols. 1971. Plant Lipid Biochemistry. Academic Press, London and New York. 387.
2. Galliard, T. 1968. Aspects of lipid metabolism in higher plants. II. The identification and quantitative analysis of lipids from the pulp of pre- and post-climacteric apples. *Phytochemistry*. **7:** 1915–1922.
3. Mazliak, P. 1965. Les lipides de l'avocat (*Persea americana* var. *Fuerte*). I. Composition en acides gras des diverses parties du fruit. *Fruits*. **20:** 49–57.
4. Davenport, J. B., and S. C. Ellis. 1959. Chemical changes during growth and storage of the avocado fruit. *Aust. J. Biol. Sci*. **12:** 445–454.
5. Kikuta, Y. 1968. Lipid metabolism in the fruit of *Persea americana*. I. Studies on the chemical composition of lipids and their changes during fruit development and storage. *J. Fac. Agr. Hokkaido Univ*. **55:** 469–495.
6. Mazliak, P., A. Ben Abdelkader, and A. M. Catesson. 1967. Biosyntheses comparee des lipides par les cellules entieres ou les mitochondries isolees du parenchyme de pomme. *Physiol. Vég*. **5**(**3**)**:** 237–260.
7. Douce, R., T. Guillot-Salomon, C. Lance, and M. Signol. 1968. Etude comparee de la composition en phospholipides de mitochondries et de chloroplastes isoles de quelques tissus vegetaux. *Bull. Soc. Fr. Physiol. Veg*. **14:** 351–373.
8. Biale, J. B., S. F. Yang, and A. A. Benson. 1966. Lipids in plant mitochondria. *Federation Proc*. **25:** 405. (Abstr.)
9. Spencer, D., and S. G. Wildman. 1962. Observations on the structure of grana-containing chloroplasts and a proposed model of chloroplast structure. *Aust. J. Biol. Sci*. **15:** 599–610.
10. Hobson, G. E., C. Lance, R. E. Young, and J. B. Biale. 1966. Isolation of active subcellular particles from avocado fruit at various stages of ripeness. *Nature*. **209:** 1242–1243.
11. Fleischer, S., G. Rouser, B. Fleischer, A. Casu, and G. Kritchevsky. 1967. Lipid composition of mitochondria from bovine heart, liver, and kidney. *J. Lipid Res*. **8:** 170–180.
12. Kodama, J. K., W. M. Butler, T. W. Tusing, and F. P. Hallett. 1963. Iothalamate: a new intravascular radiopaque medium with unusual pharmacotoxic inertness. *Exp. Mol. Pathol. Suppl*. **2:** 65–80.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1961. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **193:** 265–275.
14. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem*. **226:** 497–509.
15. Mackinney, G. 1941. Absorption of light by chlorophyll solutions. *J. Biol. Chem*. **140:** 315–322.
16. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem*. **28:** 1756–1758.
17. Gardner, H. W. 1968. Preparative isolation of monogalactosyl and digalactosyl diglycerides by thin-layer chromatography. *J. Lipid Res*. **9:** 139–141.
18. Ferrari, R. A., and A. A. Benson. 1961. The path of carbon in photosynthesis of the lipids. *Arch. Biochem. Biophys*. **93:** 185–192.
19. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*. **28:** 350–356.
20. Marinetti, G. V. 1962. Chromatographic separation, identification, and analysis of phosphatides. *J. Lipid Res*. **3:** 1–20.
21. Barr, R., M. D. Henninger, and F. L. Crane. 1967. Comparative studies on plastoquinone. II. Analysis for plastoquinones A, B, C and D. *Plant Physiol*. **42:** 1246–1254.
22. Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem*. **38:** 514–515.
23. Gerstl, B., L. F. Eng, R. B. Hayman, and P. Bond. 1969. The lipids of mitochondria of human gray and white matter. *Lipids*. **4:** 428–434.
24. Kirk, J. T. O., and R. A. E. Tilney-Basset. 1967. The Plastids. W. H. Freeman, London and San Francisco. 608.
25. Park, R. B., and N. G. Pon. 1963. Chemical composition and the substructure of lamellae isolated from *Spinacea oleracea* chloroplasts. *J. Mol. Biol*. **6:** 105–114.
26. Lyons, J. M., T. A. Wheaton, and H. K. Pratt. 1964. Relationship between the physical nature of mitochondrial membranes and chilling sensitivity in plants. *Plant Physiol*. **39:** 262–268.
27. Schwertner, H. A., and J. B. Biale. 1967. Phospholipid and glycolipid patterns in plant mitochondria. *Plant Physiol*. **42**(**Suppl.**)**:** 15.
28. Ongun, A., and J. B. Mudd. 1968. Biosynthesis of galactolipids in plants. *J. Biol. Chem*. **243:** 1558–1566.
29. Baker, J. E., L.-G. Elfvin, J. B. Biale, and S. I. Honda. 1968. Studies on ultrastructure and purification of isolated plant mitochondria. *Plant Physiol*. **43:** 2001–2022.