Lipid Transformations in Plastids of Bean Leaves and Pepper Fruits [1, 2]

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A few reports have appeared concerning the synthesis of plastid lipids. Stumpf and James (17) and Stumpf et al. (18) found that both saturated and unsaturated fatty acids may be formed from acetate by chloroplast preparations. However, in some blue-green algae, Bloch et al. (4) found that long-chain monounsaturates are synthesized by oxidative desaturation of corresponding saturates. Kates (9) suggested that C^{14} incorporation into the galactose moiety of galactolipids occurs by the conversion of glucose to UDP-glucose and by the subsequent formation of UDP-galactose. Apparently the galactolipids are rapidly labeled with C^{14} during photosynthesis with $C^{14}O_2$, whereas phosphatidyl glycerol is less rapidly labeled (3). Further, some details are known about the in vitro breakdown of plastid lipids or the presence of enzymic systems which cause the breakdown of plastid lipids (7,16,21). However, few investigations have been concerned with the in vivo degradation of plastid lipids or with the lipids of senescent plastids. Two tissues in which plastid breakdown may occur were selected for this study. Mature leaves grown under a 20 hour per 24 hour photoperiod were placed in continuous darkness following which plastids were isolated. The plastids from ripening pepper fruits were also isolated. For comparison, the plastid lipids of leaves subjected to 3 different lengths of darkness and plastid lipids from pepper fruits at 3 stages of maturity were investigated.

Materials and Methods

Plant Material. Plastids were isolated from primary bean leaf lamina of *Phaseolus vulgaris* L. var. Vaughn's Bountiful green pod bush beans subjected to varying periods of darkness. In addition, plastids were isolated from fruits of *Capsicum annum* L. var. grossum (L.) Sendt. picked from a local truck farm. Plastids were isolated separately from 2 batches of green peppers, from 2 batches of red peppers, and from one batch of intermediate stage peppers.

Methods. Approximately 350 g of primary bean leaf lamina were removed and placed in cold water for 30 minutes, weighed while wet, and ground in a blendor in 2 × volume of ice-cold, 0.35 M NaCl. After filtering through 3 layers of cheesecloth the material was centrifuged for 2 minutes at 200 × *g* at 10°. The supernatant fraction was centrifuged at 1000 × *g* for 10 minutes to sediment the chloroplasts. The sediment was resuspended in 75 ml of ice-cold, 0.35 M NaCl and recentrifuged at 200 × *g* for 2 minutes. This supernatant material was centrifuged at 1000 × *g* for 10 minutes in order to sediment the plastids. Subsequently the sediment was suspended in a small volume of 0.35 M NaCl. An aliquot of the chloroplast suspension was removed for chlorophyll analysis (10) and the remaining material was boiled in 60 % ethanol to stop further enzymic degradation of the lipids. The pepper fruit plastids were sedimented between 500 and 1000 × *g* in 0.5 M NaCl, otherwise the isolation procedure was similar to that used for bean plastid isolation. The lipids were extracted with a 2:1(v/v) chloroform to methanol mixture (6) and fractionated in a silicic acid column (Bio-Rad, minus 325 mesh, 18 g per column) using a stepwise addition of increasing concentrations of methanol in chloroform. This chromatography provided a means for separating most of the troublesome chlorophylls from the other lipids. In general, 40 or 50 % of each sample was used for fatty acid analyses. The remaining amount was chromatographed on silicic acid-impregnated paper. The samples used for fatty acid analyses were saponified in alcoholic KOH; the fatty acids were recovered after acidifying with 10 % H_2SO_4, were methylated with methanolic BF_3 (12), and were then chromatographed by gas-liquid chromatography using a 5- or 10-ft column of 12.5 % or 20 % diethylene glycol succinate on acid washed Chromosorb W (60/80 mesh) or on unwashed fire brick (60/80 mesh). The column temperature was 200° or the temperature was programed from 150 to 220°.

Standard mixtures of fatty acid methyl esters of known compounds were used to calibrate the detector

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[5] Abbreviations used: G-gal, monogalactosyl glycerol; G-gal-gal, digalactosyl glycerol; GP, glycerophosphate; GPC, glycerophosphoryl choline; GPE, glycerophosphoryl ethanolamine; GPG, glycerophosphoryl glycerol; GPI, glycerophosphoryl inositol; GPS, glycerophosphoryl serine.

Table I. *Percentages of Major Bean Chloroplast Lipids*

Treatment Hours of darkness	GP lipids	GPI lipids	GPG, GPC, GPE lipids	Unknown P	Sulfo-lipid	G-gal* lipids	G-gal-gal* lipids	Other glyco-lipids	Unknown gal
12.0 h	7.2	15.5	54.4	23.0	17.0	25.8	4.0	36.8	16.4
78.5 h	19.4	16.0	52.2	12.4	12.8	25.8	8.0	20.2	33.2
95.1 h	24.1	13.0	35.5	27.4	14.3	9.3	3.8	36.0	36.6

* The actual amounts of these compounds probably are higher than indicated in the table since the chromatographic separations were difficult and any glycolipid sugar of questionable origin is reported in the column "other glycolipids."

response. The remaining material of each combined fraction was chromatographed on silicic acid-impregnated paper (11). Each sample was divided into 3 equal portions, spotted separately on a wide sheet of paper, and developed with diisobutyl ketone-acetic acid-water (40: 25: 5, v/v). The chromatogram was then cut lengthwise to separate the simultaneously chromatographed spots; one strip was used for staining with Rhodamine 6G in order to locate the lipid components. Corresponding areas were removed from the other paper strips, eluted with 25 % chloroform in methanol (v/v), and used for phosphorus and sugar analyses (11, 1).

Results

The percentages of predominant plastid lipids are given in tables 1 and 3. Glycerophosphoryl glycerol-[5], GPC-, and GPE-lipids were at times somewhat difficult to resolve by paper chromatography and hence are reported as a group in table I.

The amount of glycerophosphate increased in the dark-grown bean plastids as opposed to the light-grown chloroplasts. This increase seemed to vary with the length of the dark period but may have been the result of phosphatidase activity during the plastid isolation. The phosphatidyl inositol content remained about the same in both the light- and dark-grown plant materials. The amount of the phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl glycerol group decreased in the dark-grown materials. However, the high concentration of this group of lipids was due to the presence of phosphatidyl glycerol. The galactolipid found in highest concentration was monogalactoglyceride, the concentration of which decreased appreciably in chloroplasts kept in the dark.

Results of the fatty acid analyses of the light- and dark-treated plastids are given in table II. As reported earlier by Debuch (5) and Wolf et al. (20), palmitic and linolenic acids were found to be the major saturated and unsaturated fatty acids. Generally, increased exposure to darkness resulted in relatively larger percentages of palmitic acid.

Accompanying the morphologic changes in pepper fruit plastids, as they develop from green to red plastids, are modifications of the major lipid components. The lipid containing GPG was the predominant phospholipid (table III); significant amounts of

Table II. *Fatty Acid Composition of Bean Chloroplasts*

Treatment Hours of darkness	Per cent								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	16/18	Saturates/ unsaturates
12.0 h	2.5	13.2	9.2	2.8	1.6	3.2	64.7	0.31	0.23
78.5 h	...	17.4	4.4	2.7	1.6	1.0	54.6	0.37	0.62
95.1 h	...	15.2	7.2	3.2	2.7	1.3	70.5	0.29	0.23

Table III. *Percentages of Components per Total Lipid Recovery in Each Isolate of Pepper Fruit Plastids*

The first batch of green peppers (1) was picked August 4, 1962; the second batch (2) was picked on September 11, 1962. The red peppers were picked on September 8 (3) and September 15, 1962 (4). The intermediate stage peppers were picked on September 6, 1962.

Fruit color	GP lipids	GPG lipids	GPE lipids	GPC lipids	GPI lipids	Other glyco-lipids	Sulfo-lipids	G-gal-gal lipids	G-gal lipids	Total* moles × 10^8	Chlorophyll (a + b) mg/isolate
Green (1)	2.4	23.6	3.2	8.2	1.8	1.8	7.1	12.0	39.9	198.6	2.59
Green (2)	5.5	22.6	8.1	16.6	2.3	1.1	5.4	11.9	26.5	115.4	5.88
Intermediate	10.0	35.6	8.0	15.3	16.0	4.1	3.1	5.2	2.7	96.1	8.64
Red (3)	0.9	35.4	2.6	14.6	1.6	4.2	5.7	6.4	28.6	234.4	0.0
Red (4)	0.0	27.7	4.9	49.4	11.0	0.0	3.8	1.5	1.8	136.6	0.0

* Isolated from 1700 to 2100 g of fruit wall material.

Table IV. *Fatty Acid Composition of Pepper Fruit Plastids*

Fruit color	Per cent								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Green (1)	2.9	2.3	23.1	3.8	6.4	11.2	31.8	16.4	2.1
Green (2)	trace	0.4	27.3	2.5	10.0	5.7	32.6	22.8	
Intermediate	0	1.8	25.8	0.4	3.2	6.3	34.1	28.5	
Red (1)	3.5	9.1	21.2	trace	4.1	6.9	30.6	24.7	
Red (2)	3.6	6.9	20.8	trace	4.3	3.1	31.7	29.7	

other phospholipids were present in the pepper plastids studied. Red plastids as compared to the green plastids exhibited a general increase of lipids containing GPG. The percentages of major glycolipids found in green pepper fruit plastids agree with those Wintermans (19) found in beet chloroplasts. Wintermans also measured the glycolipid concentrations in yellow and green Sambucus leaves. He reported a significant decrease in the major sugar-containing lipids (G-gal and G-gal-gal) of yellow, as opposed to green, leaves. Reductions in the percentage of these major sugar-containing lipids were also observed in red, as opposed to green, pepper fruit plastids. The less abundant sugar-containing lipids (such as sulfolipid) did not appear to be significantly less in amount in the red pepper plastids. Again, these results are in agreement with those of Wintermans. The major lipids of the intermediate pepper fruit plastids did not appear to be in intermediate amounts between those found in the green and those in the red pepper plastids.

A comparison of the fatty acids of pepper fruit plastids is given in tables IV and V. The ratio of saturated to unsaturated fatty acids in the pepper plastids was not dependent on the state of maturation of pepper fruits. There was a definite change, however, in the ratios of 14/16, 14/18, and 16/18 fatty acids. As opposed to bean leaf plastids, the major unsaturated fatty acid was linoleic instead of linolenic. Plastids from the 2 different batches of green peppers did not contain identical percentages of the different lipids. The same was true for the plastids from the 2 batches of red peppers. These differences may have been due to the fact that the batches were picked at different times.

Discussion

At the present state of knowledge it is difficult to relate the biochemistry of aging pepper fruit plastids to that of dark-grown leaf plastids. However, plastids subjected to darkness and those from red pepper fruits contained relatively less G-gal-lipids as compared to light-grown plastids and plastids from green fruits. Kates (8) indicated that the galactose moiety has a relatively rapid turnover rate and hence the galactose concentration varies with the metabolic state of the plastid (2). The decrease in the GPG-, GPC-, GPE-lipid group in the bean plastids was expected since phosphatidyl glycerol is the predominant phosphatide and is also actively metabolized. The general increase in the ratio of saturated to unsaturated fatty acids and of 16/18 fatty acids in the dark-, as opposed to the light-grown plastids was in agreement with those of Newman (13, 14). Rosenberg (15) found that the fatty acids of etiolated cells of *Euglena gracilis* were predominantly saturated whereas the fatty acids of cells grown in the light were mainly unsaturates. However, the ratio of saturated to unsaturated fatty acids in pepper fruit plastids did not change as green fruits changed to red fruits. It is apparent that the metabolic relationships among these compounds are complex.

Table V. *Ratios of Saturates/Unsaturates, 14/16, 14/18, and 16/18 Fatty Acids of Pepper Fruit Plastids*

Fruit color	Saturates/ unsaturates	14/16	14/18	16/18
Green (1)	0.58	0.08	0.03	0.41
Green (2)	0.57	0.01	0.01	0.50
Intermediate	0.44	0.07	0.03	0.36
Red (1)	0.61	0.43	0.14	0.32
Red (2)	0.55	0.33	0.10	0.30

Summary

The plastid lipids of bean leaves and pepper fruits were investigated to determine which compounds change as the plastids undergo morphologic change. For comparison the plastid lipids of leaves subjected to 3 different lengths of darkness and plastid lipids from pepper fruits at 3 stages of maturity were extracted following plastid isolation. Lipids were separated by silicic acid column, silicic acid-impregnated paper, and gas-liquid chromatography. Analyses were made for phosphorus, sugars, and fatty acids. Plastids subjected to long periods of darkness and those from red pepper fruits contained relatively less monogalactosyl glycerol-lipids as compared to light-treated plastids and plastids from green fruits. Increased dark treatment generally caused an increase in the ratio of saturated to unsaturated fatty acids, whereas plastids from red and green pepper fruits had the same ratio of saturated to unsaturated fatty acids.

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