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LIPIDS OF PROPLASTIDS AND NITROGEN-DEFICIENT CHLOROPLASTS*

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Abstract—The influence of darkness and a nitrogen-deficient nutrient medium, conditions which would reduce the photosynthetic rate, on the plastid lipids was studied. The galactolipids and associated fatty acids, mainly linolenic, were present in relatively less concentration in dark-treated and nitrogen-deficient plastids. Increased exposure to light caused an increase in monogalactosyl glyceride† and digalactosyl glyceride. Younger tissues were obviously more severely affected by nitrogen deficiency.

INTRODUCTION

THE work of Benson[1] and of other investigators indicates that chloroplast lipids in general are actively metabolized. Further, the concentration of each plastid lipid is very much dependent upon the previous environmental conditions to which the plastid was exposed prior to isolation and extraction. A great number of reports have appeared concerning the isolation, characterization, and synthesis of many of the chloroplast lipids. Few have dealt with the influence of environmental factors other than light on the state of the chloroplast lipids.

The most concentrated chloroplast lipids of higher plants are the galactosyl diglycerides[1] which seem to increase with increasing exposure to light.[2] The plastid galactolipids contain, as esterified fatty acid materials, almost exclusively linolenic acid,[3] the concentration of which is obviously also increased with increased exposure to light.[4] In *Euglena* cells, dark treatment results in a reduction of galactolipids and linolenic acid.[5,6] Apparently plastids can synthesize some of the lower-chain fatty acids in the dark, but galactolipids and the C_{18} unsaturated acids are synthesized mainly during exposure to light. Therefore, factors which would reduce the rate of photosynthesis, such as reduced light[7,8] and manganese[9] or iron[10] deficiency in the growing medium, should cause a reduction of the galactolipids and of the predominant fatty acid, linolenic acid.

Many of the reports of the influence of light and other environmental factors on lipids of photosynthetic cells have been concerned with whole cells of *Euglena*. The following is a report of an effort to determine the relative changes in plastid lipids of higher plants upon

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† *Abbreviations used:* GPG, glycerophosphoryl glycerol; G-gal, monogalactosyl glycerol; G-gal-gal, digalactosyl glycerol.

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exposure to different light periods and following growth in a nitrogen-deficient medium. Both treatments, darkness and nitrogen deficiency, would be expected to reduce the capacity of the photosynthetic structure to synthesize specific plastid lipids.

RESULTS

Plastids were isolated from primary leaves of 14-day-old bush bean plants grown in total darkness, in total darkness except for a final 36-hr light period, or for 14 days on a 20-hr photoperiod (about 1500 ft-candle). The relative amounts of plastid fatty acids from those leaves are given in Table 1. The relative amounts of palmitic, stearic, oleic, and linoleic acids

TABLE 1. MOLE PERCENTAGES AND TOTAL AMOUNTS OF FATTY ACIDS FROM BEAN LEAF PLASTIDS

Treatment	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total μM of Me ester kg* wet wt.
Dark	2·0	4·0	34·0	5·0	11·0	15·0	15·0	15·0	147·0
36 hr light	1·0	3·0	32·0	4·0	7·0	13·0	11·0	28·0	184·0
260 hr light	1·0	2·0	18·0	5·0	3·0	5·0	4·0	61·0	410·0
260 hr light†	1·0	5·0	18·0	5·0	2·0	3·0	2·0	64·0	810·0

* kg wet wt. of leaf material.
† Plastids were isolated from plants grown at two different times for a period of 260 total light hours.

declined upon increased light exposure whereas the relative amount of linolenic increased significantly. Consequently, the ratios of saturated to unsaturated acids, and of C_{16} to C_{18}

TABLE 2. RATIOS OF SATURATED TO UNSATURATED FATTY ACIDS, OF C_{16} TO C_{18} FATTY ACIDS, AND OF STEARIC TO C_{18} UNSATURATED ACIDS OF BEAN LEAF PLASTIDS

Treatment	Saturated:unsaturated	C_{16}:C_{18}	Stearic:C_{18} unsaturated
Dark	1·02	0·70	0·24
36 hr light	0·79	0·61	0·13
260 hr light	0·31	0·31	0·04
260 hr light	0·34	0·33	0·03

fatty acids decreased for plastids exposed to a longer period of light (Table 2). As might be expected the plastids from plants given 260 hr of light contained a significantly greater amount

TABLE 3. PHOSPHOLIPID, GLYCOLIPID, AND CHLOROPHYLL MATERIAL OF BEAN LEAF PLASTIDS

Treatment	μM Phospholipids kg wet wt	μM lipid sugar (as mono- and digalactosyl glycerides) kg wet wt.	μM galactolipid: μM phospholipid	mg Chl* *a* kg wet wt	mg Chl *b* kg wet wt.
Dark	20·1	49·5	2·5	·	—
36 hr light	32·8	6·9	0·2	46·0	17·0
260 hr light	—	—	—	241·0	87·0
260 hr light	38·9	419·0	10·8	145·0	50·0

* Chl—chlorophyll.

TABLE 4. MOLE PERCENTAGES AND TOTAL AMOUNTS OF FATTY ACIDS FROM PLASTIDS OF SQUASH PLANTS GROWN ON COMPLETE OR NITROGEN-DEFICIENT NUTRIENT MEDIUM

Treatment	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total μM of Me ester/kg wet wt.
−N, 1st node	0·8	2·8	35·6	2·2	2·7	3·3	2·3	50·3	370·0
Complete, 1st node	0·2	1·1	22·8	5·2	3·6	8·3	4·0	55·0	142·0
−N, 2nd node	0·0	2·7	17·7	3·0	3·9	5·4	4·2	63·0	226·0
Complete, 2nd node	0·8	1·4	9·6	1·4	0·8	2·1	2·3	81·8	385·0

of galactosyl glycerides than plastids of dark-grown plants. However, there did not seem to be such a large increase in the lipid phosphorus (Table 3). Since the plastid isolation was not quantitative, values of relative changes in the plastid lipids would be more significant than values of absolute changes.

TABLE 5. RATIOS OF SATURATED TO UNSATURATED FATTY ACIDS, OF C_{16} TO C_{18} FATTY ACIDS, AND OF STEARIC TO C_{18} UNSATURATED ACIDS OF PLASTIDS FROM SQUASH PLANTS GROWN ON COMPLETE OR NITROGEN-DEFICIENT NUTRIENT MEDIUM

Treatment	Saturated/unsaturated	C_{16}/C_{18}	Stearic/C_{18} unsaturated
−N, 1st node	0·72	0·64	0·05
Complete, 1st node	0·38	0·39	0·05
−N, 2nd node	0·32	0·27	0·05
Complete, 2nd node	0·14	0·13	0·01

Squash plants were grown in hydroponic tanks containing either a complete mineral nutrient medium or a nitrogen-deficient, but otherwise complete, nutrient medium. Plastids were isolated from first and second node leaves when the second node leaves of the nitrogen-deficient plants were very yellow. The nitrogen-deficiency apparently prevented synthesis

TABLE 6. PHOSPHATIDYL GLYCEROL, GALACTOLIPID, AND CHLOROPHYLL CONTENT OF NORMAL AND NITROGEN-DEFICIENT SQUASH LEAF PLASTIDS

Treatment	μM GPG-lipids/ kg wet wt.	μM phospholipids/ kg wet wt.	μM G-gal-lipids/ kg wet wt.	μM G-gal-gal-lipids/ kg wet wt.	μM Galactolipid/ μM GPG-lipid	mg Chl *a*/ kg wet wt.	mg Chl *b*/ kg wet wt.
−N, 1st node	49·7	91·3	101·0	85·7	3·8	131·0	47·0
Complete, 1st node	41·4	62·2	197·0	128·0	7·9	70·0	23·0
−N, 2nd node	—	1·7	4·4	—	—	146·0	49·0
Complete 2nd node	—	4·4	51·6	—	—	172·0	56·0

of normal amounts of linolenic acid (Table 4). The ratios of saturated to unsaturated acids and of C_{16} to C_{18} fatty acids were greater for nitrogen-deficient plastids because of the reduced linolenic acid in the nitrogen-deficient plastids. It is interesting that younger leaves had a lower ratio of saturated to unsaturated acids (Table 5). Older tissue plastids seem to contain

less linolenate than younger tissue plastids.[4] Plastids from older leaves of nitrogen-deficient plastids seem to have contained relatively reduced amounts of monogalactosyl glyceride but not of phosphatidyl glycerol (Table 6).

DISCUSSION

Stumpf and James[11] have shown that isolated lettuce-leaf plastids are capable of synthesizing long-chain fatty acids and complex lipid components. However, the conversion of oleic → linoleic → linolenic has not been established for isolated plastids and yet can be accomplished by whole leaves. Even with the application of a full complement of cofactors, isolated higher-plant plastids showed a diminished rate of fatty acid synthesis in the dark. Exposure to light increased acetate incorporation 1·7 to 2·4 times.[12] Concomitant with increased fatty acid synthesis during increased exposure to light is the appearance of an increased concentration of monogalactosyl glyceride (Table 3). The results reported here are in agreement with those found by Stumpf and James,[11] Wintermans,[2] and Newman.[4] It is interesting that increased light exposure causes an increase in the C_{18} unsaturated acids but not a great increase in the C_{16} unsaturated one. These results do not definitely demonstrate an oleic → linoleic → linolenic acid conversion in the plastids. However, there does seem to be some suggestion of this sequence since the relative concentrations of oleic and linoleic acids decreased and that of linolenic acid increased upon exposure to light. It must also be pointed out that the relative concentration of stearic acid decreased upon exposure to light; however, Stumpf and James[13] have clearly demonstrated the absence of a significant conversion of stearic to oleic acid in isolated lettuce-chloroplast preparations. Other work with *Euglena* would tend to substantiate much of the above information.

Another attack made upon the problem of the relationship between photosynthetic capacity and the accumulation of chloroplast lipids was to withhold nitrogen from the mineral nutrient and subsequently analyze the plastid lipids of nitrogen-deficient tissues. While Pankov[14] has suggested that nitrogen starvation decreases the amount of leaf phosphatides, little is known concerning the influence of nitrogen on the chloroplast lipids. As might be expected, nitrogen deficiency also reduced the relative amount of plastid glycolipid and linolenate. The treatment seemed to have a greater effect on the concentration of G-gal-lipids than on G-gal-gal-lipids and GPG-lipids. Younger leaves were more severely affected by the nitrogen deficiency since, under the conditions used, it was suspected that nitrogen was not mobilized to any great extent from the older to the younger leaves.

EXPERIMENTAL

Plastids were isolated from bush bean (*Phaseolus vulgaris* L. var. Burpee's Tender Pod) or squash (*Cucurbita maxima* Duchesne var. Burpee's Blue Hubbard) leaves. The bean plants were grown for 14 days in the dark, for 12·5 days in the dark followed by a final 36-hr light period (1460 ft-candle), or for 14 days on a 20-hr photoperiod (1460 ft-candle). The squash plants were started in vermiculite, irrigated with distilled water, and then irrigated with either a complete mineral nutrient medium or a nitrogen-deficient, but otherwise complete, nutrient. The squash plants also were grown under 20-hr photoperiods (1460 ft-candle) for 22 days prior to harvest.

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Plastids were isolated by differential centrifugation in cold 0·35 M NaCl. The fraction sedimenting between 200 and 1000 *g* was collected, washed, and recentrifuged. The fraction sedimenting between 200 and 1000 *g* was again collected and used as the plastid fraction. The sediment was made to volume in cold 0·35 M NaCl, an aliquot was taken for chlorophyll analysis, and the remainder was boiled in 60% aqueous ethanol to inactivate the phosphatidases. Chlorophylls were analyzed by the method of Koski.[15]

Lipids were extracted with 2:1 (v/v) chloroform:methanol,[16] washed, dried under nitrogen, and chromatographed in a 15 g silicic acid column (Bio-Rad silicic acid, minus 325 mesh).[17] Most of the troublesome chlorophylls and carotenoids were eluted with chloroform and the glycolipids and phospholipids were eluted with increasing concentrations of methanol in chloroform. The chloroform–methanol and methanol fractions were divided. Part of each fraction was used for fatty acid analysis. The remaining part of each fraction was chromatographed on silicic acid-impregnated paper with diisobutyl ketone:acetic acid:water (40:25:5, v/v)[18] followed by chromatography in thin layers of silica gel (10% $CaSO_4$ as binder) developed with diisobutyl ketone:acetic acid:water (40:25:3, v/v).[19] The compounds were eluted from the layer and analyzed for lipid phosphorus[20] and sugar.[21,22] Small amounts of lipids separated on silicic acid-impregnated paper were also co-chromatographed with known compounds in thin layers developed with chloroform:methanol:water (85:25:3, v/v) as an aid in identification.

For the fatty acid analyses, the lipids were saponified in alcoholic KOH; the fatty acids were extracted with diethyl ether and petroleum ether after acidification with 10% aqueous H_2SO_4, and were then methylated with methanolic BF_3. The fatty acid methyl esters were analyzed by gas chromatography on a 10 ft DEGS column (15% liquid phase on acid-washed Chromosorb W). Some fatty acid methyl esters were collected following chromatography, brominated, and rechromatographed as a further aid in identification. NIH standard mixtures were used to calibrate the detector and determine retention times. Suspected unsaturated acids were also isomerized and the u.v. absorption spectra of these compounds were determined.

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