Changes in Lipid Composition during Greening of Etiolated Pea Seedlings

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ABSTRACT

After 7 days of germination in the dark, the three sections of pea seedlings studied (cotyledons, stems, and young leaves) are rich in linoleic acid; after illumination of the seedlings a very significant increase in linoleic acid is observed in the young leaves section, whereas only small variations are noted in the fatty acid composition of the other sections. The increase in linoleic acid results from the increase in galactolipid content of the young leaves; these already linoleic acid-rich galactolipids are present but only in small amounts in the etiolated seedlings (10% of total lipid).

Variations in composition of the other lipid classes (phospholipids and neutral fats) were also studied. The possibility of fatty acid transport from the cotyledons toward the young leaves during the synthesis of the photosynthetic apparatus is discussed.

Variations in lipid metabolism as related to greening and formation of the photosynthetic apparatus have been studied mainly in algae, Chlorella and Euglena (5, 12–14). But it is known that the greening of higher plants differs markedly from that of photosynthetic algae. Whereas algae pass from a heterotrophic metabolism to an autotrophic or semiautotrophic metabolism during greening and this transformation is largely reversible, in higher plants the etiolated seedlings subsist only at the expense of food reserves made by the plant, and greening is essential for its survival and presents an irreversible feature. Moreover, although the lipid compositions of green algae and higher plants have common characteristics such as high levels of galactolipids with polyunsaturated fatty acids and presence of phosphatidylglycerol with trans-3-hexadecenoic acid (7), appreciable differences are encountered between them: (a) in algae, the galactolipid polyunsaturated fatty acids consist of 16 and 18 carbon chain fatty acids, whereas in higher plants, γ-linolenic acid alone accounts for 80% of the galactolipid fatty acid (7); (b) in higher plants, the turnover of the fatty acid portion of galactolipids is very low while the 14CO2 incorporation into the galactose portion is very rapid (16).

The few studies on the lipid metabolism of etiolated barley (2) or clover leaves (17) have shown that during greening the incorporation of acetate-1-14C into linolenic acid does not clearly indicate how this fatty acid is synthesized as the chloroplast matures. We may assume that linolenic acid is not mainly formed de novo, but from an endogenous precursor already present in the etiolated seedling (perhaps from another fatty acid present in large quantities in the cotyledon). However, the low incorporation of radioactive tracers in the fatty acid portion does not permit us to define further the metabolic pathways of the galactolipid biosynthesis.

In this study, we have followed the changes in fatty acid composition of each lipid class during the greening of the etiolated pea seedlings (in which the cotyledons and the young leaves are clearly cut) in order to elucidate the biosynthetic pathways of chloroplast galactolipids as well as the action of light on the formation of these lipids, and to show the relationship between storage lipids and structural lipids during greening.

MATERIAL AND METHODS

Pea seeds (Pisum sativum L. var. Progrès de Laxton), were purchased from W. H. Perron’s seed company, Chomedey, Quebec.

For germination and greening studies, seeds were planted in flats and placed in the dark for 7 days. The seedlings were then rinsed under tap water, placed on absorbent pads, and greened at room temperature under fluorescent lights (type F 40-Gro Sylvania) for 0, 24, 36, 48, or 96 hr.

Lipid Analysis. Pea seedlings, 30 g, were gathered and cut into sections corresponding to cotyledons, stems (epicotyls), and young leaves (plumules), for separated lipid analyses. Tissues were fixed in boiling water and then extracted exhaustively with chloroform–methanol (1:1, v/v) according to the method of Bligh and Dyer (4).

Lipid extracts were fractionated into classes by combined column and thin layer chromatography. Neutral lipids were separated on standard 20- × 20-cm chromatoplates coated with a 250-μ layer of Silica Gel G with the hexane–ether–acetic acid (80:20:2, v/v) solvent mixture as eluant. Galactolipids were eluted in acetone–acetic acid–water (100:2:1, v/v) solvents according to Gardner (6), in whose system phospholipids do not migrate but galactolipids are well resolved. Phospholipids are first eluted from silicic acid (Bio-sil HA, 325 mesh, Bio-Rad Laboratories) column according to Vorbeck and Marinetti (18), after removal of neutral lipids with chloroform and galactolipids with acetone, and further fractionated by thin layer chromatography in chloroform–acetone–methanol–acetic acid–water (5:2:1:1:0.5, v/v) solvents (9). Characterization and identification of each class of components were performed by one- or two-dimensional thin layer chromatography with the aid of specific spray reagents as described earlier (8, 17).

Each lipid class was separated by preparative thin layer chro-
matography for further analysis of their fatty acid portion. After development of the chromatoplates in the solvent systems mentioned above, zones of interest were visualized under ultraviolet light after Rhodamine 6 G sprays, scraped off the plate, and eluted with chloroform–methanol–acetic acid–water (5:4:1:0.1, v/v); after evaporation of solvents, lipid residues were analyzed for fatty acids as in the following.

Fatty acids from each class were methylated by the boron trifluoride (0.5 ml 14% boron trifluoride in methanol, Applied Sciences Laboratories) method according to Metcalfe, Schmitz, and Pelka (10). Fatty acid methyl esters were analyzed by gas-liquid chromatography using a Pye-Unicam, series 104, model 64, apparatus equipped with a dual flame detector and 5-ft x 4-inch columns filled with 5% diethylene glycol succinate on 100-120 mesh Gas Chrom Q; column temperature was maintained at 165°C, detectors and injectors at 200°C, and helium flow rate at 40 ml/min. Standard mixtures (Applied Science Laboratories) of known fatty acid methyl esters were used for comparison of retention times. Quantitation of fatty acids were accomplished by the triangulation method and by the addition of methyl heptadecanoate as internal standard. The use of this internal standard permitted also quantitation of each lipid class, in multiplying the fatty acid value by the appropriate factor for each lipid analyzed.

RESULTS AND DISCUSSION

Changes in Lipid Content of the Various Seedlings. Each section of the seedling varies in weight during the greening. Cotyledons, the major portion of the seedling, decrease; the stem increases slightly and then remains stable whereas the weight of the young leaves increases steadily during greening (Fig. 1A).

The three sections differ also in lipid contents. After 7 days of germination in the dark, cotyledons contain 5 mg of fatty acid per g of fresh weight; leaves, 2.7 mg; and stems, 0.7 mg. These fatty acid contents remain fairly constant in the stem and the young leaves during the greening, but they decrease markedly in the cotyledons. As Figure 1C shows, the loss in lipid content of cotyledons per 100 g fresh weight during the 96-hr illumination is 10 times greater than the gain in the lipids of the young leaves.

Variations in Fatty Acid Composition. Variations in total fatty acid composition are shown in Figure 2. The most important changes occur in the young leaves in which decreases in linoleic acid and increases in linolenic acid are observed. Among the other fatty acids, only palmitic acid shows percentage decreases. As expressed in mg of fatty acid per 100 g total seedling, linoleic acid first increases, then remains unchanged. Coincidentally, it is also in the leaves that the chlorophyll biosynthesis is active. On the other hand, the stem, which is rich in linoleic acid, does not show significant changes in fatty acid composition.

Cotyledons show increase in linoleic acid and decrease in oleic acid from 20% to 10% after 96 hr of illumination, while their lipid content markedly decreases.

The increase in linolenic acid content of the young leaves during greening may result from a synthesis de novo. However, from our previous observation with clover (17) it seems that this synthesis de novo is not the major route of linolenic acid production during the formation of the photosynthetic apparatus. We therefore considered whether cotyledons, which lost a considerable amount of lipids during greening, would not participate in the formation of galactolipids, as, for example, in translocation of fatty acids to the leaves where they could be desaturated to form linolenic acid. This significant loss of lipids in the cotyledons noted during the illumination was also observed during seed germination. One may think that it accounts in great part for the β-oxidation of fatty acids or their utilization in the glyoxylate cycle (3). On the other hand, since the decrease in lipid content of cotyledons is 10 times greater than the uptake of lipids in the

Fig. 1. Greening of the etiolated seedling after 7 days of germination in dark. A: Changes in fresh weight of various sections; B: changes in lipid content of various sections; C: changes in lipid content per 100 g fresh weight of seedlings. Seedlings were placed 40 cm from the fluorescent lights.
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FIG. 2. Changes in content and composition of fatty acids in the various sections of seedlings during greening. ● C16:0:palmitic acid; □: C16:1:palmitoleic acid; △: C18:0:stearic acid; ■: C18:1:oleic acid; ○: C18:2:linoleic acid; ◊: C18:3:linolenic acid.

FIG. 3. Changes in lipid classes of young leaves during greening.

leaves, it is very likely that a translocation mechanism is masked by the more abundant lipid degradation. The only fatty acid which shows a significant percentage decrease in the cotyledons in the course of greening is oleic acid. The weight of oleic acid lost in cotyledons during the 96-hr illumination period amounted to 70 mg/100 g fresh weight of seedling, which is large enough to account for the corresponding increase in linolenic acid in leaves (25 mg/100 g fresh weight of seedling).

Another observation which supports this hypothesis is the fact that oleic acid accumulates in the stems and leaves under certain conditions of germination in dark. When these seedlings (with no less than 30% of oleic acid in stems and leaves) are illuminated, oleic acid is converted into linoleic and linolenic acids.

Changes in Lipid Composition of Young Leaves. In the young leaves, the percentage of phospholipid slightly decreases at the beginning of the illumination, falls deeply between 24 and 72 hr, then seems to stabilize. The increase in digalactosyldiglyceride is regular and that of monogalactosyldiglyceride is greatest between 36 and 72 hr. The ratio of monogalactolipid to digalactolipid is in good agreement with the generally observed ratio of about 2 in mature chloroplast (1, 15-17), a characteristic of the active photo-

(same conditions as in Fig. 1). PL: Phospholipids; MGDG: monogalactosyldiglyceride; DGDG: digalactosyldiglyceride; NL: neutral lipids.
Table I. Neutral Lipid Content and Their Fatty Acid Composition in Young Leaves with Various Times of Illumination

The seedlings were illuminated after 7 days of germination in the dark.

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<th>Neutral Lipids</th>
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synthetic lamellae. On the other hand, the neutral lipid content slightly decreases (Fig. 3).

Prior to illumination both galactolipids are very rich in linolenic acid (Fig. 4). The percentage of this fatty acid in the galactolipids only slightly increases during greening. On the other hand, because of the important increase in weight of the two galactolipids, the linolenic acid content per 100 g of total fatty acid increases considerably in the leaves. All other fatty acids are only present in small quantities in these two lipids.

Neutral lipids amount to not more than 10% of total lipid in the young leaves, but they show important variations in fatty acid composition (Table I). Among neutral lipids, free fatty acids are much more saturated than triglycerides and diglycerides.

In the total phospholipids, changes in fatty acid composition are minor; a slight decrease is observed in the linoleic content which is by far the most abundant fatty acid in the phospholipids; the decrease in total linoleic acid is due mainly to a decrease in phospholipid content rather than a change in the fatty acid pattern (Fig. 5, A and B).

Among the phospholipids (Table II), phosphatidylcholine is the most abundant and is very rich in linoleic acid, and the relative percentage of this fatty acid only slightly decreases. Phosphatidylethanolamine is initially about half as abundant as phosphatidylcholine and is also rich in linoleic acid, the latter remaining stable during the 96-hr illumination. Phosphatidylglycerol does not increase markedly during the 96-hr illumination but shows increased palmitic acid content after the first 24-hr...
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Fig. 5. Changes in the fatty acid composition in the phospholipids of young leaves during greening (same conditions and same abbreviations and symbols as in Fig. 1).

illumination, although part of this fatty acid might be accounted for by trans-3-hexadecenoic acid, which is normally found in phosphatidylglycerol of green leaves. The other phospholipids are poorer in linoleic acid.

The sulfolipid, although present in the leaves as shown by twodimensional thin layer chromatography, was not analyzed for its fatty acid composition.

As a result, it seems likely that the decrease in phospholipids arises from the enlargement of the chloroplast compartment, characterized by a larger amount of galactolipids, at the expense of the extrachloroplast compartment, which is rich in phospholipids.

The fact that the phospholipid constituents decrease in weight without changing their fatty acid pattern does not clearly indicate that phospholipids are involved in the synthesis of galactolipids by transesterification. Rather, the presence of diglycerides rich in linoleic and linolenic acids permits one to postulate that these glycerides may play a role as precursors of galactolipids (11).

In addition, the almost complete absence of linolenic acid among the free fatty acids of young leaves excludes the possibility of translocation of this fatty acid as free in this section of the seedling.

From the present studies, it is also evident that light is not required for the formation of linolenic acid of the galactolipid molecules, since linolenic acid-rich galactolipids already exist in the etiolated seedling, prior to illumination. Light, however, stimulates quantitative production of galactolipids either directly on their biosynthesis chain or indirectly by providing energy for the formation of an abundant galactose pool capable of stimulating galactosylation.

The presence of galactolipids always rich in linolenic acid permits one to assume that desaturation of the diglyceride precursors is closely related to galactosylation.

Acknowledgments—This work was partially done in the Departments of Food Science and Plant Science. We wish to thank these departments for assistance and laboratory facilities.

LITERATURE CITED


Table II. Phospholipid Contents and Their Fatty Acid Composition in Young Leaves with Various Times of Illumination

<table>
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1 Palmitic acid, trans-Δ2-hexadecenoic acid?