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Light-dependent Induction of Polyunsaturated Fatty Acid Biosynthesis in Greening Cucumber Cotyledons[1]

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ABSTRACT

Greening cucumber (*Cucumis sativus* L.) cotyledons exhibited dramatic increases in the ability to desaturate exogenously added [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid within 2 to 3 hours of illumination. These increases were effectively inhibited by 10 micrograms per milliliter cycloheximide. Oleate desaturation remained at a high level in constant light for 5 to 6 days after induction and then declined by about 50%; when returned to the dark, the tissue showed a sharp decrease in conversion of [^{14}C]oleate to [^{14}C]linoleate. Linoleate desaturation reached a maximum about 15 hours after induction and declined immediately thereafter while the tissue still was in the light; after induction had peaked return of the tissue to the dark showed a dramatic fall of linoleate desaturation. The changes in desaturation were correlated with the conversion of the principal fatty acid in the etiolated cotyledons, linoleate, to α-linolenate, and with the assembly of the chlorophyll-containing photosynthetic membranes. The incorporation of [1-^{14}C]acetate into lipids showed no significant light stimulation. The role of light in the regulation of certain aspects of plant metabolism during development is discussed.

Polyunsaturated fatty acids, esterified to phospholipids or galactolipids, are the principal components of the lipid matrix of plant membranes (12). In particular, they are concentrated in the photosynthetic membranes of chloroplasts where the most unsaturated fatty acid, α-linolenic acid, can account for up to 90% of the total fatty acid in the organelle (17). The generation of the fatty acid precursor, acetyl-CoA, from photosynthetically fixed CO_2 can occur in intact isolated chloroplasts from spinach leaves (18–21). The *de novo* synthesis of palmitoyl-ACP[2] from acetyl-CoA also occurs in the chloroplast, as does the subsequent elongation and desaturation to oleoyl-ACP (16, 27). There are indications from both avocado mesocarp (11, 33) and spinach leaf systems (16, 18, 27) that the chloroplast is the principal site of saturated and monoenoic C_{18} fatty acid synthesis in these tissues. However, both the subcellular location(s) and the enzymic mechanism(s) of the final stages in C_{18} polyunsaturated fatty acid biosynthesis remain unresolved (9).

Isolated chloroplasts have been shown to be incapable of synthesizing significant amounts of polyunsaturated fatty acid to date (16); the principal product of *in vitro* incubations with fatty acid precursors is oleate, either in free or esterified form (6, 18, 27). It has been proposed that further desaturation of oleate occurs in an extrachloroplastic location, possibly in a microsomal fraction, where the acyl substrate is esterified to phosphatidyl choline (6, 9, 25). In addition, hexadecatrienoic acid (16:3 [7, 10, 13]) has been implicated as a precursor of α-linolenic acid in leaf tissue (15). There are a number of technical problems associated with the study of polyunsaturated fatty acid formation in leaves. Among the most formidable of these is the apparently very low rate of desaturation observed *in vivo* (23) which makes reliable *in vitro* studies very difficult. Another serious difficulty is the rapidity of degradation of free polyunsaturated fatty acids by plant enzyme systems (7). Polyunsaturated fatty acids are known to turn over relatively slowly in the mature leaf (30).

One promising approach is the use of greening plant tissue previously grown in the dark. The onset of greening in such etiolated tissue is known to be associated with an increase in the levels of α-linolenic acid (1, 9, 14, 28, 32). In this paper we report the induction of the desaturation stages of polyunsaturated fatty acid biosynthesis by light in greening cucumber cotyledons. The induction of the desaturation (*i.e.* conversion of oleic to linoleic and hence to α-linolenic) is dependent upon protein synthesis and shares a number of characteristics with other light-induced processes in plants.

MATERIALS AND METHODS

Plant Material. Cucumber seeds, *Cucumis sativus* L. var. Alpha green, were the gift of the Niagara Chemical Division, F.M.C. Corporation, Modesto, Calif. The seeds were soaked for 20 min in a saturated solution of the fungicide Botran (Tuco Division of Upjohn Co., Kalamazoo, Mich.). A 5-cm layer of Vermiculite in a darkened baking tray was moistened to saturation with distilled H_2O and covered with filter paper. The seeds were spread evenly over the surface of the moist filter paper and covered with a darkened lid which was sealed with black tape. The seedlings were routinely germinated in complete darkness for 6 days at 22 C.

Reagents and Substrates. [1-^{14}C]Acetate (58 Ci mol^{-1}), [1-^{14}C]oleic acid (56 Ci mol^{-1}), and [1-^{14}C]linoleic acid (60 Ci mol^{-1}) were purchased from Amersham/Searle. Ethylene glycol monoethyl ether was obtained from Sigma Co. All solvents were reagent grade.

Light Treatment. The developing seedlings were normally illuminated by exposure to fluorescent white light (G.E. F15T8D, Daylight, 15 w) of intensity (quantum flux) <200 μE m^{-2} sec^{-1} (about 4.8 mw cm^{-2}). Light intensity was measured with a Quantum radiometer model LI 185A (Lambda Instrument Corp.). Variations of both intensity and nature of the light source are discussed under "Results."

Chl Determinations. Cotyledons were dried with filter paper to remove surface moisture, weighed, and extracted several times in 80% acetone using a TenBroek ground glass homogenizer. The extract was filtered, diluted to 0 to 0.2 *A* units, and measured at 663 and 645 nm in a Gilford model 240 spectrophotometer. Chl concentrations were calculated according to the method of Arnon (2).

Incubation of Tissue. Cotyledons were detached and surface moisture removed with filter paper. For incubations with aqueous

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[2] Abbreviations: ACP: acyl carrier protein; PC: phosphatidylcholine.

substrates, the cotyledons were gently abraded with small razor cuts on their upper surfaces and placed in 2 ml incubation mixture containing the ^{14}C-labeled substrate in a 0.1 M phosphate buffer at pH 6.5. Nonpolar substrates, *i.e.* [1-^{14}C]oleate and [1-^{14}C]linoleate, were purchased dissolved in benzene or hexane; the volatile solvent was removed with a stream of N_2 at room temperature and the dried substrate was then redissolved in a similar quantity of ethylene glycol monomethyl ether. The substrates were applied directly to the upper surfaces of abraded cotyledons, which were then placed in 2 ml of 0.1 M phosphate buffer at pH 6.5. All incubations were performed under aerobic conditions with gentle shaking in a water bath at 22 C in a stoppered 25-ml Erlenmeyer flask under various conditions of illumination.

Lipid Extraction and Analysis. Reactions were terminated by the removal of the tissue from the incubation medium followed by its immediate extraction in either chloroform-methyl alcohol, 2:1 (v/v) or hexane-isopropyl alcohol 3:2 (v/v) (10). In both cases, the plant tissue (typically two to three cotyledon pairs) was homogenized thoroughly in a TenBroek ground glass homogenizer in the presence of about 20 ml/g of the appropriate solvent. The residues were repeatedly extracted and the extracts combined. Chloroform-methyl alcohol extracts were purified by the partitioning of aqueous contaminants in 0.1 M KCl in 1% acetic acid followed by three washed in glass-distilled H_2O (19). Hexane-isopropyl alcohol extracts were purified by partitioning against 50% of their volume of 6% aqueous Na_2SO_4. The mixture was left to stand for a few min before removal of the upper hexane-rich layer containing the lipids (10). Purified lipid extracts were concentrated by evaporation of the solvents under N_2. The extraction procedures were monitored at each stage by the removal of aliquots for radioactivity determination in a PCS (Phase Combining System, Amersham/Searle) cocktail using a Beckman LS230 liquid scintillation counter.

Total lipid mixtures were resolved by one- and two-dimensional TLC on precoated Silica Gel G plates. Polar lipids were separated in chloroform-methyl alcohol-water, 65:25:4 (v/v), chloroform-methyl alcohol-acetic acid-water, 85:15:10:3.5 (v/v), or acetone-acetic acid-water, 100:2:1 (v/v). Neutral lipids were separated in acetone-benzene-water, 91:30:8 (v/v), petroleum ether-diethyl ether-acetic acid, 80:20:1 (v/v), or benzene-diethyl ether-ethyl acetate-acetic acid, 80:10:10:0.4 (v/v) (18). Separated lipids were identified by comparison with authentic standards in several solvent systems and by the use of specific spray reagents.

^{14}C-Labeled lipid bands were located on one-dimensional plates by means of a Packard model 7201 radiochromatogram scanner and the bands scraped into vials for liquid scintillation counting. For the detection of ^{14}C-labeled lipid spots on two-dimensional plates an autoradiographic technique was employed. The developed plates were exposed to Kodak C-Omat XR-5 x-ray film for 2 days to 2 weeks and the x-ray film subsequently developed in an appropriate x-ray developing system.

Methyl esters of total lipid mixtures or separated lipids were prepared by saponification at 80 C for 1 hr in 2 N KOH, followed by extraction into petroleum ether, and esterification at 0 C with diazomethane. The methyl esters were separated on a 10% DEGS-PS (Supelco, Bellefonte, Pa.) column fitted to a Varian aerograph model A-90-P gas chromatograph equipped with a thermal conductivity detector. Radioactivity of the GLC peaks was monitored continuously with a Nuclear-Chicago Biospan model 4998 radioactive detector.

Identity of Fatty Acid Products. Preparations from young pea leaves have been shown to convert either [1-^{14}C]- or [U-^{14}C]linoleic acid to a C_{12} monoenoic (Δ^9) dibasic acid which may co-chromatograph with linolenic acid when derivatized and analyzed on GLC (31). For this reason the fatty acid products of the [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid incubations were checked to ensure their authenticity.

Methyl esters were purified by preparative TLC on Silica Gel G in benzene. The band corresponding to a methyl ester standard contained >95% of the radioactivity. Following elution of the purified methyl esters, they were separated by GLC and gave an identical labeling pattern to the original unpurified mixture. As a further check, the methyl ester mixture was separated by development in hexane-diethyl ether, 80:20 (v/v) on Silica Gel H plates containing 5% $AgNO_3$. Bands corresponding to saturated, mono-, di-, and trienoic methyl ester standards were scraped off the plates and applied to the GLC. The results confirmed that all of the incubation products were authentic methyl esters of C_{18} fatty acids.

The position of the first double bond with reference to the ^{14}C label in the reaction products was checked by reductive ozonolysis (26). Since [1-^{14}C]oleic and [1-^{14}C]linoleic acids were the substrates, it was expected that the reaction products would also display a double bond at the C_{9-10} position with ^{14}C label in the C_1-C_9 fragment after ozonolysis, if a desaturation occurred toward the methyl end of the substrate. After reductive ozonolysis of the purified reaction products of [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid incubations, only the C_9 aldehyde ester was labeled. We concluded that [1-^{14}C]oleic had been converted to [1-^{14}C]linoleic and [1-^{14}C]linoleic had been converted to [1-^{14}C]linolenic by a single desaturation of the appropriate substrate.

RESULTS

Growth of Etiolated Seedlings and the Effect of Illumination. The cotyledons of cucumber seeds are primarily storage organs and, during the course of the germination of the seeds, substrates are exported from them at a rapid rate to support the growth of roots and stem. This was reflected by a sharp decline in the dry weight of the cotyledon tissue during the 1st week of development and a slower decline after that (Fig. 1). There is a corresponding increase in dry weight of the newly formed stem and root systems and the dry weight of the plant as a whole fell by less than 20% during the first 2 weeks of development. Illumination of dark-grown plants affected only the dry weight of the cotyledons which, after a delay of about 10 hr, began to increase sharply. This reflected the increase in protein synthesis and the onset of CO_2 fixation and photosynthetic electron transport in the newly green-

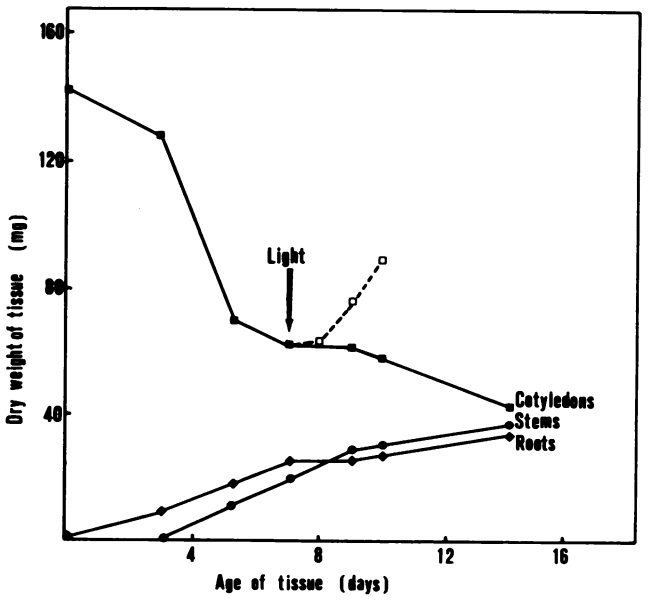

FIG. 1. Changes in the dry weight of various parts of developing etiolated cucumber seedlings. (■——■): Dark-grown cotyledons; (●——●): dark-grown stems; (◆——◆): dark-grown roots; (□- - -□) cotyledons following illumination on day 7 (no other tissues were affected by illumination within the time scale of this experiment).

ing tissue. The cotyledons continued to serve as the main photosynthetic organs of the plants for at least 3 to 4 weeks after greening during the development of the primary leaves.

Changes in Fatty Acid Composition of Cotyledons during Development. Dark-grown cucumber cotyledons showed relatively little change in their fatty acid composition during at least the first 10 days of development (Table I). The principal acyl component of the tissue was $\Delta^{9,12}$ *cis*-octadecadienoic acid (linoleic acid), which comprised 60 to 70% of the total fatty acids. There were no traces of very long chain ($>C_{20}$) fatty acids at any stage of development, and only trace quantities of short chain fatty acids ($<C_{14}$). The dramatic changes in the metabolism of the cotyledons following illumination were paralleled by a large increase in the proportion of $\Delta^{9,12,15}$ *cis*-octadecatrienoic acid (α-linolenic acid). During this time the net dry weight of the plants did not increase by more than 10% and the amount of lipid present increased only in proportion to this figure. Therefore, the substantial decrease in the percentage of linoleic acid and the concomitant increase in the percentage of α-linolenic acid probably reflects the desaturation of the former to the latter. There was very little change in the combined proportions of these fatty acids, which stayed at about 70%, as in the etiolated tissue. The proportions of the other principal fatty acids did not change markedly during greening.

Effect of Temperature upon Fatty Acid Composition during Greening. Since only the C_{18} polyunsaturated fatty acids showed large and consistent changes during greening, the data for the other fatty acids are omitted from Figure 2. This figure shows the effect of greening on the C_{18} polyunsaturates at three different growth temperatures reflecting the range encountered by the plant during growth. At the lowest temperature, 15 C, the cotyledons failed to germinate. After 24 hr at 20 C to permit germination, the plants were returned to the 15 C regimen and their fatty acid compositions monitored. Figure 2a shows a slight increase in the proportion of α-linoleic acid during development at 15 C, but no effect when the plants were illuminated. This was paralleled by a failure of the cotyledons to green to any significant extent. Growth of both the stem and root systems was also much reduced both before and after illumination compared to plants grown at higher temperatures.

Plants grown at 22 C (Fig. 2b) exhibited a steady rise in the proportion of α-linolenic acid in the dark, although this was greatly accelerated once the plants were illuminated. Both these plants and those grown at 35 C showed normal development of the shoot and root systems and the typical greening response to illumination. Plants grown at 35 C made less α-linolenic acid in the dark than did those at 22 C (Fig. 2c). However, once they were illuminated, the cotyledons rapidly converted the bulk of their pool of linoleic acid to α-linolenic acid. As with plants grown at 22 C the most rapid conversion of linoleic to α-linolenic acid took place over the initial 10 hr of greening, with a much reduced rate after this. By the 3rd day of greening α-linolenic acid constituted 40 to 50% of the total fatty acid in the cotyledons compared to a value of about 60% in light-grown tissue. Thus the conversion was substantially completed by this stage.

Uptake of ^{14}C-labeled Fatty Acids by Cotyledons. Oleate and linoleate desaturation was assayed under *in vivo* conditions by incubating detached cotyledons with the appropriate ^{14}C-labeled

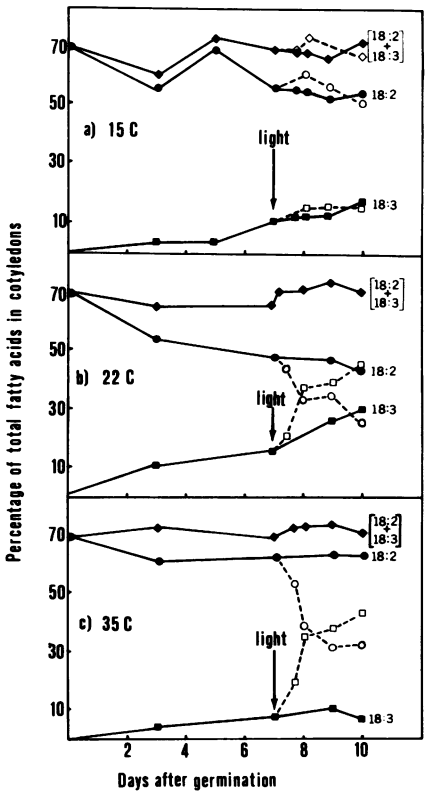

FIG. 2. Changes in proportions of linoleic acid (18:2) and α-linolenic acid (18:3) during development of etiolated and greening cucumber cotyledons grown at: (a) 15 C; (b) 22 C; (c) 35 C. (——): Etiolated tissue; (- - -): illuminated tissue.

Table I. Fatty Acid Composition of Developing Etiolated and Greening Cucumber Cotyledons.

	Time After Germination (Days)	Percentage of Total Fatty Acids					
		16:0	18:0	18:1	18:2	18:3	18:2+18:3
Dark-grown plants	0	13.9	6.4	10.5	69.2	tr	69.2
	3	15.6	6.3	13.7	60.2	4.1	64.5
	7	14.9	6.1	10.4	62.4	6.1	68.5
	9	14.2	5.7	7.4	62.6	10.1	72.7
	10	12.6	6.8	11.0	63.0	6.5	69.5
Dark-grown plants illuminated after seven days	7 + 10 hr*	15.8	6.7	6.0	52.7	18.8	71.5
	7 + 1 day	17.0	4.6	4.9	37.6	34.6	72.2
	7 + 2 days	16.7	5.5	10.0	31.1	36.8	67.9
	7 + 3 days	15.8	3.6	5.8	32.4	42.3	74.5

*Second number refers to duration of greening. The plants were grown at 34 C.

substrates. Over 90% of the added [1-^{14}C]oleic acid was taken up by the tissue in 1 hr. The uptake of [1-^{14}C]linoleic acid was slower but at least 50% of the added substrate had entered the tissue during the normal assay period of 4 hr.

Stimulation of Desaturation by Light. Dark-grown cotyledons were only capable of relatively low rates of [1-^{14}C]oleic acid desaturation and little or no α-linolenate was formed under these conditions. Following a period of illumination, their capacity for desaturation of [1-^{14}C]oleic acid to both [^{14}C]linoleate and α-[^{14}C]linolenate increased markedly. The intensity of the light had relatively little effect on the increase in desaturation in the range of light intensities employed here (Fig. 3).

Induction of Desaturation by Preillumination. The apparent induction of the ability to desaturate both [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid was assayed following the exposure of cucumber cotyledons to a range of different preillumination times. In both cases there was relatively little desaturation in the absence of preillumination (Fig. 4). Very short preillumination times (<40 min) had little effect. Following this lag period, increasing preillumination times resulted in correspondingly increased desaturation activities up to 8 to 10 hr when there was some leveling off of both activities. Therefore, a standard length of 10-hr preillumination was employed in subsequent experiments.

Time Course of Desaturation. The kinetics of the desaturation of added [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid are shown in Figure 5. In both cases the desaturation was greatly stimulated by a 10-hr period of preillumination in white light. Both "low" and "high" light intensities (8 and 186 μE m^{-2} sec^{-1}, respectively) gave almost identical effects on the subsequent activities of both desaturations. Desaturating activity was much reduced in the absence of preillumination, although this was more marked in the case of [1-^{14}C]linoleic acid conversion to [^{14}C]linolenic acid. The actual assays were performed in the dark and thus the apparent stimulation of desaturation by preillumination is not merely the consequence of increased uptake of the substrate or a direct effect of light on the desaturation process. The uptake of the substrates was the same in both etiolated and greening cotyledons. The rate of [1-^{14}C]oleic acid desaturation reached a maximum after 2.5 to 3 hr and then fell off sharply with time. For this reason the length of most assays were standardized at 4 hr. The desaturation of [1-^{14}C]linoleic acid was relatively slow compared with [1-^{14}C]oleic acid and the rate did not fall off as sharply after 4 to 5 hr. This may have been due to the slower uptake of [1-^{14}C]linoleic acid which would have resulted in a slower but more even release of the [^{14}C]substrate into the linoleate pool.

Inhibition of Desaturation by Cycloheximide. Cycloheximide, which inhibits protein synthesis of eukaryotic cells, was effective in halting the light-mediated induction of both desaturations (Fig. 6). In the case of [1-^{14}C]oleic acid desaturation there was a lag period of 2 hr before the inhibitor affected the activity. This delay may have been due to a relatively slow uptake of the inhibitor during the early stages of the incubations. The inhibition of any further increase in [1-^{14}C]oleic acid desaturation by cycloheximide was not accompanied by any discernible reduction in desaturation, which suggests a relatively stable enzyme(s) with a low turnover rate.

In both experiments cotyledons were detached and placed in

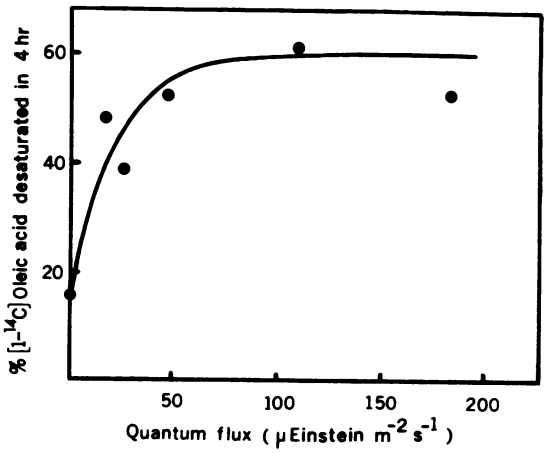

FIG. 3. Effect of light intensity upon desaturation of [1-^{14}C]oleic acid by greening cucumber cotyledons. Cotyledons were greened at different light intensities indicated for 10 hr and subsequently incubated with [1-^{14}C]oleic acid in the dark for 4 hr.

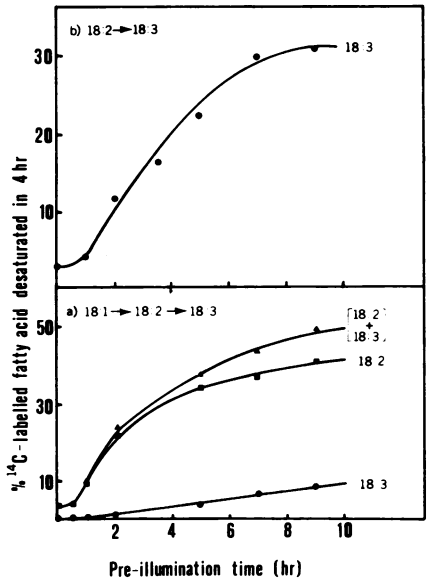

FIG. 4. Effect of preillumination upon: (a) [1-^{14}C]oleic acid and (b) [1-^{14}C]linoleic acid desaturation in greening cucumber cotyledons. All assays were performed for 4 hr in the dark.

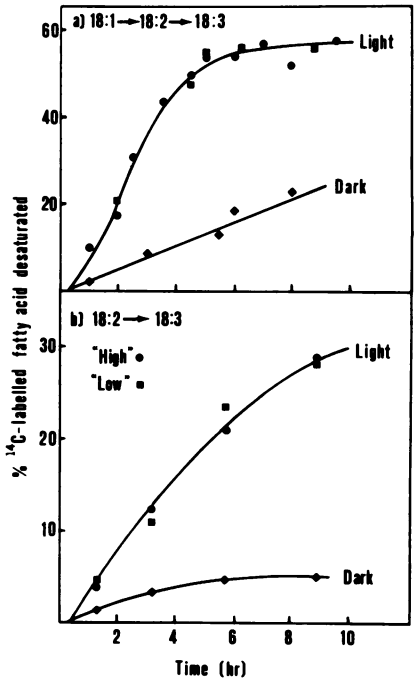

FIG. 5. Time course of desaturation of: (a) [1-^{14}C]oleic acid and (b) [1-^{14}C]linoleic acid by etiolated and greening cucumber cotyledons. Greening cotyledons were preilluminated for 10 hr in white light at either "high" (186 μE m^{-2} sec^{-1}) or "low" (8 μE m^{-2} sec^{-1}) light intensity. All assays were performed in the dark.

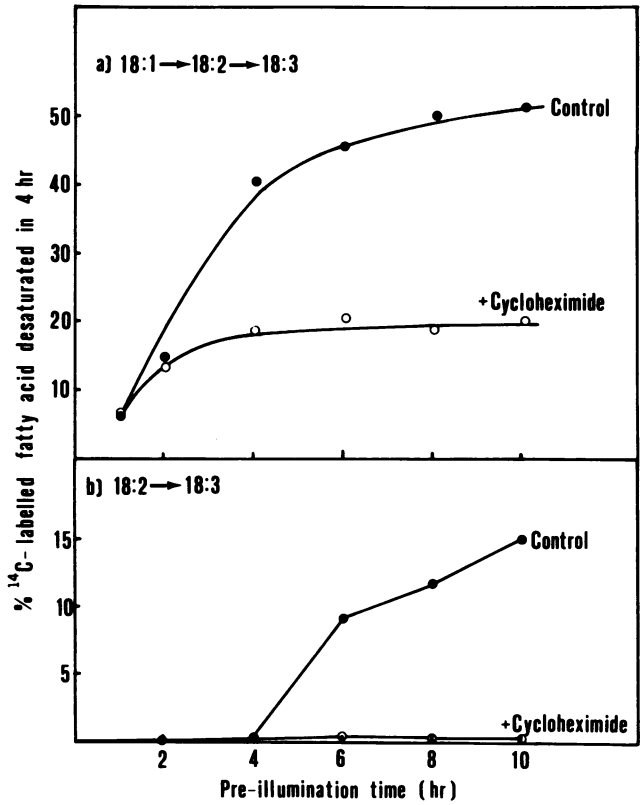

FIG. 6. Inhibition by cycloheximide of light-induced desaturation in greening cucumber cotyledons. Detached cotyledons were floated in buffer solution ± 10 μg ml^{-1} cycloheximide during preillumination. All assays were performed for 4 hr in darkness.

buffer solution before illumination and greening started. The 4-hr lag in onset of ability to desaturate added [1-^{14}C]linoleate found in the experiment described in Figure 6b was not observed in other experiments (*i.e.* Fig. 4); in all of these experiments illumination was started while the cotyledons were still attached to the seedling. Cycloheximide supplied in the experiment of Figure 6b entered the cotyledons during the lag period; complete inhibition had occurred prior to the time the controls began to demonstrate an increase in activity. The ability of cycloheximide at 10 μg/ml to inhibit cotyledon protein synthesis was checked in simultaneous experiments using [U-^{14}C]leucine (34).

Simultaneous Induction of Chl Biosynthesis and Polyunsaturated Fatty Acid Biosynthesis by Illumination. The exposure of etiolated cucumber seedlings to light resulted in the rapid induction of Chl biosynthesis in the cotyledons (Fig. 7). Under conditions of constant illumination, Chl was produced at a linear rate in the tissue for 30 to 35 hr. After this, Chl synthesis continued but at a much reduced rate, and the concentration of Chl in the cotyledons declined slightly. This decline was due to the rapid expansion of the cotyledons after the first 2 to 3 days, when they began to take on the appearance and photosynthetic functions of true leaves. The exposure of etiolated seedlings to a more natural diurnal light regimen resulted in a similar rise in Chl levels during illumination followed by a decline during the dark period (data not shown). Under such conditions the final peak Chl concentration achieved by the cotyledons was slightly higher than in continuously illuminated seedlings, but the time required to reach this value was significantly longer under the diurnal light regimen. The slight drop in Chl concentrations during the diurnal dark period was examined further by returning plants that had greened for about 24 hr to the dark and monitoring the levels of Chl in the cotyledons (Fig. 7). These plants lost Chl over the next few days ($T_{1/2}$ = 28 hr) and 10 to 20% of the cotyledons actually reverted to the characteristic yellow color of etiolated plants. The reversibility of the process was demonstrated by reilluminating these plants after almost 6 days in the dark, whereupon they immediately began to biosynthesize Chl once again.

The results of simultaneous *in vivo* assays of oleate and linoleate desaturation during greening and the effect of returning the plants to darkness are given in Figure 8. Both activities responded to light within several hr as was noted in Figure 4. When the seedlings were maintained under constant illumination the response of the activities varied. Oleate desaturation was maintained at a fairly constant level for at least 5 days before declining to about 50% of this level by the 7th day after greening. Linoleate desaturation was already in decline by the end of the 1st day and continued to fall off steadily during the following days. The two activities also differed sharply in their response to a return to darkness after about 24 hr of illumination. Whereas linoleate desaturation rapidly fell to a level of almost zero ($t_{1/2}$ = 3.6 hr), oleate desaturation diminished only gradually over a period of a week ($t_{1/2}$ = 96 hr) and by the 8th day it was still operating at 40 to 50% of its maximal value in the light. The half-lives of these activities ($t_{1/2}$) were calculated assuming first order kinetics during the decay. The values do not necessarily represent the rate of turnover of the enzymes responsible for the desaturation reactions but suggest different stabilities in the two desaturation steps. The levels of endogenous α-linolenate, which rose rapidly during the early stages of greening (*cf.* Table I), declined again when the plants were returned to darkness after 24 hr in the light. The decline was not noticeable for the first 2 to 3 days of dark treatment and the proportion of α-linolenate had only declined by 40 to 50% after 8 days. Since linoleate desaturation was not present in the dark-treated tissue, the slow decline in α-linolenate probably reflects its known low rate of turnover (30). When the dark-treated plants were reilluminated after about 7 days they were relatively deficient in α-linolenate compared to continuously illuminated plants, and linoleate desaturating activity increased once more.

Effect of Illumination upon [1-^{14}C]Acetic Acid Incorporation into Lipids. Greening cucumber cotyledons incorporated [1-^{14}C]acetate, supplied at a concentration of 17 μM, at an initially rapid rate and then at the lower rate until about 25% of the total [1-^{14}C]acetate had been taken up after 10 hr (Fig. 9). Etiolated cucumber cotyledons incorporated [1-^{14}C]acetate at about 80% of the rate of the light-treated plants. Both etiolated and greening cotyledons utilized 40 to 50% of the total incorporated [1-

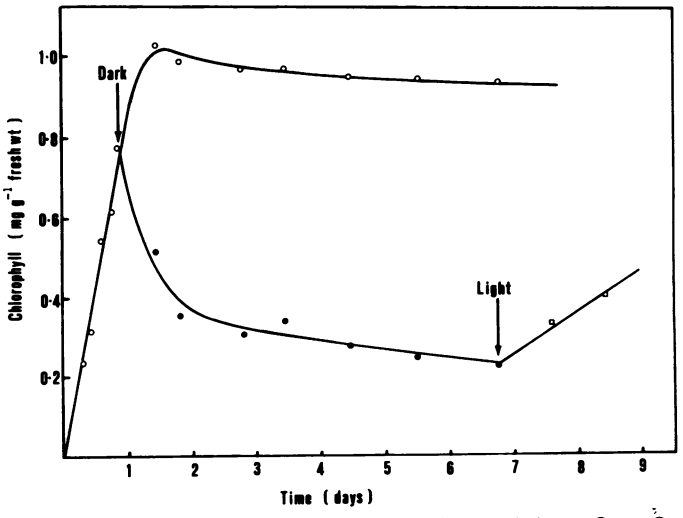

FIG. 7. Chl biosynthesis in greening cucumber cotyledons. (○——○): Seedlings under constant illumination; (●——●): seedlings returned to darkness after 24-hr illumination; (□——□): seedlings reilluminated after about 6 days in darkness.

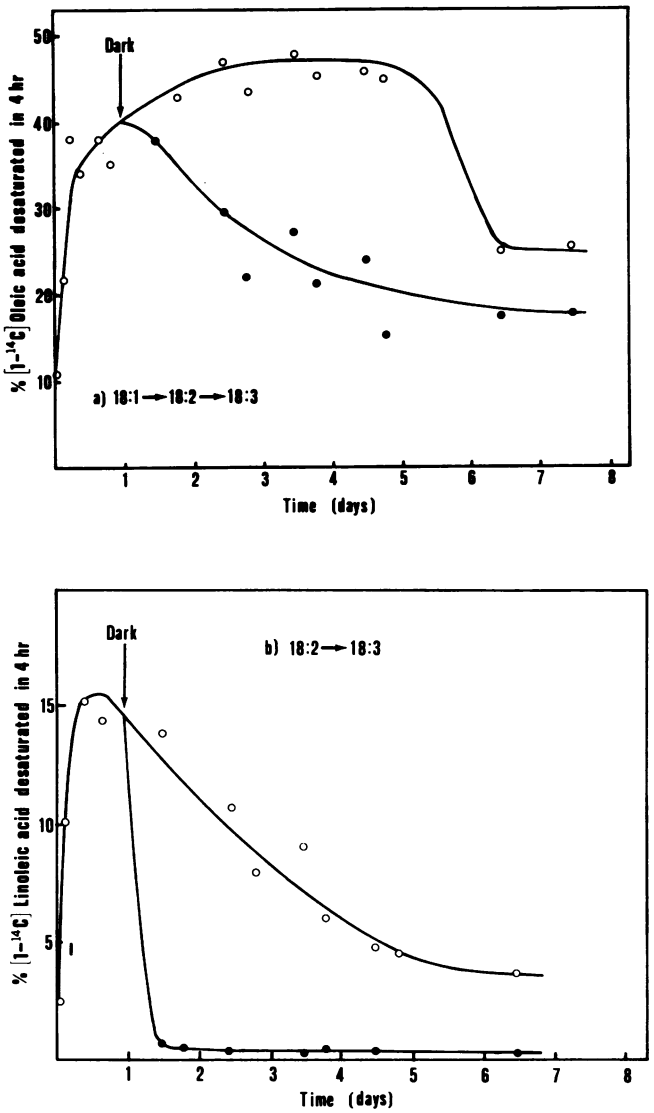

FIG. 8. Induction of oleate and linoleate desaturation in greening cucumber cotyledons in constant light and the effect of a subsequent return to darkness. (○——○): Constant light; (●——●): darkness.

^{14}C]acetate for fatty acid biosynthesis and this proportion did not vary significantly with time. The lack of any significant difference in the characteristics of [1-^{14}C]acetate incorporation into lipids in light- and dark-treated plants is demonstrated in Figure 9b. Analysis of the labeling patterns of the fatty acids from the plants confirmed the similarity of this incorporation. In both cases the principal ^{14}C-labeled fatty acid was palmitate, which accounted for 45 to 60% of the total radioactivity in the lipid fraction. Most of the remainder of the ^{14}C was in oleate (20–25%) and linoleate (5–15%). The proportion of ^{14}C in palmitate decreased with time, whereas it increased in the C_{18} fatty acids, especially the polyunsaturates. The lack of any significant difference in the characteristics of [1-^{14}C]acetate incorporation into lipids in etiolated and greening cotyledons contrasts sharply with the results from [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid (see under "Discussion").

^{14}C-Labeled Lipid Products of Incubation. The labeled fatty acids formed during [1-^{14}C]acetate incubations were mainly associated with phospholipids and very little free fatty acid was ever detected. Etiolated cotyledons incorporated label mainly into phosphatidyl ethanolamine (about 60%) and PC (about 30%). The remainder of the label was in neutral lipids such as diacylglycerols and triacylglycerols. Following a period of preillumination, the greening cotyledons incorporated <50% of the label from [1-^{14}C]acetate into PC and only a relatively small proportion (10–15%) into phosphatidyl ethanolamine. The greening cotyledons also incorporated relatively more label into neutral lipids and pigments.

[1-^{14}C]Oleic acid was incorporated by greening cotyledons as the free fatty acid and subsequently acylated both phospholipids and neutral lipids. Label appeared in PC within 30 min of the start of incubations, with the proportion rising to 30% within 1 hr and remaining at approximately this level for the next 5 hr. The amount of unesterified [1-^{14}C]oleate declined steadily with time and label accumulated mainly in neutral lipids such as di- and triacylglycerols. Analysis of the fatty acid profiles of the principal labeled lipids showed that the free fatty acid contained exclusively [1-^{14}C]oleic acid. Both PC and the neutral lipid band contained the desaturation products, linoleate and α-linolenate and the proportion of label in these increased with time.

DISCUSSION

The illumination of dark-grown cucumber cotyledons led to the rapid desaturation of the principal fatty acid present in the tissue, linoleate, to α-linolenate, which is the most common fatty acid found in light-grown cotyledons (Table I and Fig. 2). This suggests a light-dependent induction or activation of one or more of the enzymes involved in the desaturation process, which appears to be superimposed on a slow desaturation process in etiolated tissues (Fig. 5, a and b). Such a phenomenon has been reported for a

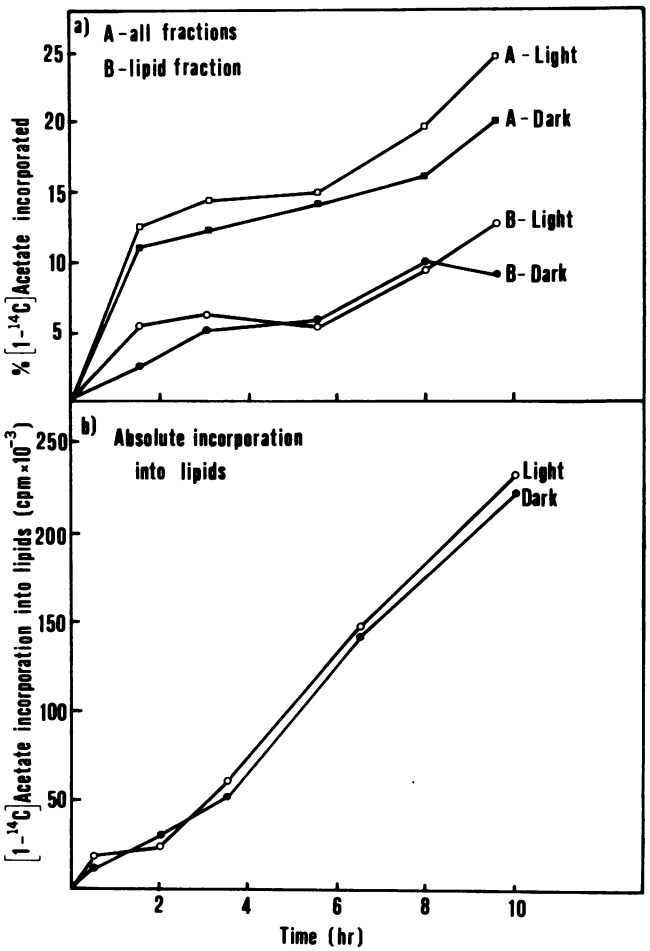

FIG. 9. [1-^{14}C]Acetate uptake and incorporation into lipids in cucumber cotyledons. (a): Proportion of [1-^{14}C]acetate supplied to cotyledons that was incorporated into: (A) all fractions; (B) lipid fraction. Light-treated plants (○ or □) were preilluminated for 10 hr prior to the incubations, which were performed for 4 hr. (b) Absolute incorporation of [1-^{14}C]-acetate into lipids in etiolated and preilluminated cotyledons.

number of other enzyme systems in greening tissue including Chl biosynthesis (3), flavenoid biosynthesis (8), and nitrate reductase (4, 22, 29). The incubation studies with [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid confirm that there is a light-dependent induction of the enzymes responsible for their desaturation. The induction process has a number of characteristics in common with the other light-induced systems mentioned above. The nature of the effect of light intensity (Fig. 3) was similar to its effect upon the induction of phenylammonia lyase in *Xanthium* leaf discs (35, 36). It has since been demonstrated that a number of other enzymes involved in both general phenylpropanoid metabolism (group I) and flavone glycoside biosynthesis (group II) are also induced in the presence of light (8).

In the present study both oleoyl and linoleoyl desaturation declined if the plants were maintained in constant light conditions, although the decline of linoleoyl desaturation was both more rapid and occurred sooner than the eventual decline of oleoyl-desaturating activity (Fig. 8). The data suggest that the enzymes may be specifically induced during the greening process in order to supply the α-linolenic acid necessary for the newly developing photosynthetic membranes. This process would be substantially completed in 1 to 2 days (Fig. 2) and thus it is not surprising to find that the bulk of the endogenous linoleate was desaturated during this time and also that the linoleoyl-desaturating activity rose to a peak and then fell off after about 15 hr.

Most light-induced enzyme systems are also sensitive to a return to dark conditions, which normally causes a decline in their activities. Both oleoyl and linoleoyl desaturation declined in the dark with linoleic desaturation once again the more labile activity (Fig. 8). Oleoyl desaturation declined relatively slowly in the dark and was still at almost 50% of its maximal light activity after 5 days. Since the principal activity *vis à vis* fatty acid metabolism in the newly greening cotyledons is linoleate desaturation to α-linolenate, it is to be expected that the linoleate-desaturating enzyme system would prove more responsive to illumination. The inhibition of the light-dependent increase in desaturating activities by cycloheximide (Fig. 6) suggests that the process is dependent upon protein synthesis on the 80S (*i.e.* cytoplasmic) ribosomes and parallels similar findings in the other light-induced systems mentioned above. The absence of a light effect on [1-^{14}C]acetate incorporation into lipids (Fig. 9), at least after 12 hr of greening, has also been observed in isolated plastids from *Hordeum vulgare* leaves, although the incorporation did eventually rise after 48+ hr of greening here (14). Acetate incorporation into fatty acids is principally an assay for the *de novo* fatty acid synthetase system which generates palmitoyl ACP, and palmitate was the main product of the acetate incubations. The results suggest that the *de novo* fatty acid synthetase is not light-induced.

These data, together with the differences in the light induction and subsequent dark inactivation of the oleoyl- and linoleoyl-desaturating systems, argue against a coordinated stimulation of fatty acid synthesis immediately following the illumination of dark-grown cucumber seedlings. Rather there appears to be a specific induction of linoleic desaturation in order to mobilize the relatively large pool of linoleate in the etiolated tissue. When the conversion to α-linolenate was completed, linoleic desaturation declined. It is possible that this process is coupled with that of Chl biosynthesis, or that they are both controlled by a common mechanism, since both activities responded to light in the same manner. In addition, the halting of Chl synthesis during the early stages of greening (by chopping the leaves into small slices or by low temperature) was found to impair seriously the induction of linoleic desaturation, while oleic desaturation was less susceptible to such treatment.

The actual subcellular location and mechanism of desaturation are a subject of some controversy at present (5, 6, 9, 24, 25). In the present studies it was noted that both [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid were rapidly incorporated into PC by greening cucumber cotyledons. The desaturated reaction products were found to be preferentially acylated to PC and a "cold chase" experiment with unlabeled substrate resulted in the loss of ^{14}C-fatty-acids from PC. These results provide indirect support for the proposed acyl-carrier role of PC during polyunsaturated fatty acid synthesis (6, 24, 25). However, they do not rule out the occurrence of the desaturation elsewhere, and the subsequent accumulation of the products on the PC molecule. These results also imply that several different enzymes are operating in the conversion of an exogenous ^{14}C-fatty-acid to α-linolenate. In addition to the actual desaturases and the carrier systems responsible for the flow of electrons into the desaturation complex for the activation of O_2, there are probably specific acyl transferases, thioesterase, and phospholipases involved in the process. Any one or more of these enzymes may be light-dependent and thus exercise control over the entire sequence of incorporation, acylation, and desaturation.

Greening cucumber cotyledons represent a good source of oleoyl- and linoleoyl-desaturating activities during a limited period of 1 and 7 days, respectively, after illumination. It seems clear that the enzymes are induced only when required for the assembly of the components of the photosynthetic membranes. Subsequent activities would be confined to keeping pace with membrane turnover, which proceeds at a much slower rate (30) and such material would not be expected to provide a good source of C_{18} desaturase activities. These observations may explain the failure to observe high desaturase activities in other *in vivo* or *in vitro* photosynthetic systems. The strict parameters of desaturation that have been defined in this investigation should now serve as a guide to the elucidation of the location and nature of the terminal desaturase reactions of polyunsaturated fatty acid biosynthesis by *in vitro* studies currently in progress in this laboratory.

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