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Development of Lipid Synthesis from CO_2 in *Avena* Leaves during Greening of Etiolated Seedlings

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The development of the lipid synthesizing system in *Avena* leaf sections was examined in connection with carbon fixation during the greening of etiolated seedlings under light. During the initial 2 h illumination there was a low level of CO_2 fixation by PEP carboxylation, but its products, malate and citrate, did not serve as a carbon source for lipid synthesis, although lipid synthesis from acetate had already been established. With the initiation of Calvin cycle activity after the initial 2 h illumination, lipid synthesis began, with CO_2 fixed by RuBP carboxylation serving exclusively as the carbon source. Fatty acid synthesis in the leaves during the initial 3 h illumination, unlike the fatty acid synthesis thereafter, was insensitive to thiolactomycin, an inhibitor of type II fatty acid synthetase contained in the plastids, and was not dependent on light, in contrast to light-dependent activity in greened leaves.

The distribution of ^{14}C incorporated into lipid molecules from $NaH^{14}CO_3$ showed an equal ratio of ^{14}C in fatty acid, glycerol and choline moieties of labeled phosphatidylcholine, but a denser radioactivity in the galactose moiety than in the residual moiety of mono- and di-galactosyldiacylglycerols. This suggests a regulated supply of glycerol, choline and fatty acid moieties for phosphatidylcholine synthesis, and an excess supply of galactose to diacylglycerol moiety for galactosyldiacylglycerol synthesis in *Avena* leaves.

Key words: Carbon fixation — Fatty acid synthesis — Greening — Lipid synthesis — Oat (*Avena sativa*) — Thiolactomycin.

Seeds germinated in the dark grow into etiolated seedlings which contain etioplasts with prolamellar bodies in their cells. When transferred to light, the etioplasts develop into etiochloroplasts and chloroplasts with stacking thylakoids. In the development of etioplasts to chloroplasts of *Avena sativa* L., the prolamellar bodies disappear gradually, concomitant with an increase in the primary thylakoids. However, there is no synthesis of membrane in the plastids during the initial 3–4 h illumination, because the total area of the membranes remains unchanged (Bradbeer et al. 1974). It is not until after this initial periods that the thylakoids proliferate markedly and stacking actually begins. The reaction centers of both photosystems I and II appear within 1 h of illumination, and their proton-pumping and photophosphorylation capacities are evident after 2 h. After the initial 3–4 h illumination, water splitting and electron transport are established, then light-dependent CO_2 fixation are initiated (Wellburn and Hampp 1979). De novo synthesis of fatty acids is also established with the beginning of photosynthesis and increases with greening (Ohnishi and Yamada 1980a).

Abbreviations: ACP, acyl carrier protein; DG, diglyceride; DGDG, digalactosyldiglyceride; FA, fatty acid(s); G3P, *sn*-glycerol-3-phosphate; MGDG, monoglactosyldiglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEP, phosphoenolpyruvate; PG, phosphatidylglycerol; PI, phosphatidylinositol; RuBP, ribulose-1,5-bisphosphate; SQDG, sulphoquinovosyldiglyceride; TLC, thin-layer chromatography.

Lipid changes during the proliferation of thylakoids and development of grana system have been reported in barley (Selldén and Selstam 1976, Tevini 1977), maize (Lürssen 1970, Mackender 1979), wheat (Bahl et al. 1976, Galey et al. 1980), pea (Roughan and Boardman 1972, Trémolières and Lepage 1971), bean (Roughan and Boardman 1972) and oat (Ohnishi and Yamada 1980a). At the initial stages (the first 3 h of illumination) of greening *Avena* leaves, lipids are partially degraded, but thereafter plastid lipids, MGDG, DGDG, SQDG and PG, increase, whereas extraplastidal lipids, PC, PE and PI, decrease. These results support the proliferation of thylakoid membranes.

Lipid synthesis requires a number of molecular constituents, such as fatty acids, glycerol and polar head group in order to proceed. The synthesis of these constituents involves different pathways and sites. Fatty acids are synthesized from acetyl-CoA in the plastid (Nakamura and Yamada 1974, Ohlrogge 1979), whereas glycerol and polar head moieties are believed to be synthesized in the extraplastid space from different precursors such as glycerol-3-phosphate, CDP-choline and UDP-galactose. Little is known about the regulatory mechanism behind the synthesis and association of these constituents. A further problem is whether these constituents are derived from photosynthetically fixed CO_2 directly or indirectly. In the case of greening rice (*Oryza sativa*) seedlings some dark carbon fixation by PEP carboxylase takes place before the development of photosynthetic carbon fixation, although the latter exceeds dark fixation with greening (Raghavendra and Das 1977).

The present paper deals with the development of the lipid synthesizing system in greening *Avena* leaves, in connection with the carbon assimilation system, and presents preliminary evidence for the mechanism regulating lipid synthesis from its constituents.

Materials and Methods

Plant material—Seeds of oat, *Avena sativa* L. var. Victory I (The General Swedish Seed Company, Limited) were germinated for 8 days in darkness at 25°C. Etiolated seedlings were then illuminated with 1,500 lux of fluorescent light (5 W m^{-2}) for 24 h and harvested at intervals. The first leaves (ca. 8 cm long) were excised at the nodes with a razor blade and cut into 2 cm sections between 4 cm and 6 cm from the leaf base.

^{14}C incorporation—Leaf sections (0.1 g) were cut into 2.5 mm pieces and dipped in 1 ml solutions of 10 mM [^{14}C]$NaHCO_3$ (100 μCi), or 0.2 mM sodium [1-^{14}C]acetate (1 μCi) in the presence or absence of thiolactomycin (4.08 μg ml^{-1}). In the other incubation, sodium [1-^{14}C]acetate was replaced by 0.2 mM L-[U-^{14}C]malic acid (1 μCi), 0.2 mM [1,5-^{14}C]citric acid (1 μCi) or 0.2 mM [U-^{14}C]glycerol (2 μCi). All incubations were carried out at 25°C for 30 min under illumination at 25,000 lux by incandescent lamps or in darkness. The leaf sections were rinsed with water and then immersed in 1 ml of 2-propanol at 80°C.

The permeation of exogenously added labeled substrates into *Avena* leaf protoplasts was confirmed by silicone oil centrifugation (Nishimura 1982) using silicone oils Toray SH-550 : SH-556 (5 : 2, v/v). The protoplasts were prepared from 12 h-greened *Avena* leaves by the method of Edwards et al. (1978) in a reaction mixture (200 μl) containing 50 mM HEPES-NaOH (pH 7.5), 0.4 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.2 mM ^{14}C-substrate (1 μCi) and 80 μg protein of protoplasts. The relative incorporation of acetate, malate and citrate at 12 min was 1 : 1.5 : 0.3.

Lipid analysis—Leaf lipids were extracted according to Bligh and Dyer (1959). The lipid extract was taken to dryness, dissolved in a small amount of chloroform and applied to silica gel TLC. Lipid samples were developed on a plate with acetone : benzene : water (91 : 30 : 8, v/v/v) for polar lipids (Pohl et al. 1970) and with petroleum ether : chloroform : acetone (4 : 1 : 1, v/v/v) for neutral lipids (Kleinig and Kopp 1978). The lipids were located under

UV light after spraying the plate with 0.01% (w/v) primuline in 80% (v/v) acetone (Wright 1971). The lipid spots were scraped off and counted with 5 ml of toluene scintillator (containing 3 g of 2,5-diphenyloxazole and 0.1 g of 1,4-*bis*[2-(5-phenyloxazolyl)]-benzene in 1 liter of toluene) in a Beckman LS 9000 scintillation system.

Acyl-CoA and acyl-ACP was determined according to Mancha et al. (1975).

For the distribution of radioactivity in lipid classes, PC, MGDG and DGDG, separated by silica gel TLC, were methanolyzed with 1 ml of 2.5% (w/w) HCl in methanol at 80°C for 2 h. Fatty acid methyl esters were extracted twice with 1 ml of petroleum ether. The remaining solution was evaporated in vacuo and hydrolyzed with 1 ml of 6 M HCl at 100°C for 6 h, concentrated in vacuo and applied to paper chromatographic analysis. The separation of glycerol, choline and glycerophosphorylcholine from the PC hydrolyzate was carried out by paper chromatography on Toyo No. 50 filter paper with 2-propanol : water : conc. ammonia (7 : 2 : 1, v/v/v) (Davidson and Stanacev 1970), and the separation of glycerol and galactose from the galactolipid hydrolyzate with *n*-butanol : pyridine : water (6 : 4 : 3, v/v/v) (Ito et al. 1974). The radioactive residues separated on the paper were located by autoradiography and determined by liquid scintillation counting.

Fixation products from [^{14}C]*NaHCO*$_3$—After incubation with [^{14}C]$NaHCO_3$, the leaf sections were rinsed in water and extracted with 2-propanol. The labeled compounds were separated by two dimensional paper chromatography on Whatman No. 1 filter paper. The solvent system was 88% (v/v) phenol : water : acetic acid : 0.2 M EDTA (831 : 154.2 : 9.8 : 5.0, v/v/v/v) in the first direction and 93.7% (v/v) *n*-butanol : 45% (v/v) propionic acid (1 : 1, v/v) in the second direction (Pedersen et al. 1966). After the paper had been dried, the radioactivity of the spots was detected by autoradiography and determined in an Aloka GM counter (GM5004).

Analysis of G3P from [^{14}C]*glycerol*—After incubation with [^{14}C]glycerol, the leaf sections were rinsed in water and extracted with 80% methanol. The extract was concentrated and developed on Toyo No. 50 filter paper with ethyl acetate : formamide : pyridine (1 : 2 : 1, v/v/v) in the first direction and ethyl acetate : acetic acid : water (3 : 3 : 1, v/v/v) in the second direction (Mortimer 1952). After the autoradiographic preparation, the radioactive G3P was eluted with 80% ethanol and determined by liquid scintillation counting.

Assay for carboxylation enzymes—The first leaves of *Avena* seedlings were ground by a mortar and pestle at 4°C, with four volumes of 50 mM Tris-HCl (pH 7.8) containing 5 mM dithiothreitol, 0.1 mM EDTA, 2 mM $MgCl_2$ and 0.5% (w/v) sodium ascorbate. The extract was filtered through two layers of Miracloth (Chicopee Mills, Inc.) and the filtrate was centrifuged at 15,000 $\times g$ for 15 min. The resulting supernatant was used as the enzyme source.

RuBP carboxylase activity was determined according to Lorimer et al. (1977). The reaction mixture (1 ml) contained 100 mM Tris-HCl (pH 8.2), 20 mM $MgCl_2$, 5 mM dithiothreitol, 0.4 mM RuBP, 20 mM [^{14}C]$NaHCO_3$ (0.5 mCi $mmol^{-1}$) and 0.02 ml of the supernatant. The supernatant was previously passed through a Sephadex G 25 column (2 $\times$ 15 cm) equilibrated with 100 mM Tris-HCl (pH 8.6) containing 20 mM $MgCl_2$, 10 mM $NaHCO_3$ and 1 mM dithiothreitol. The reaction was started by addition of RuBP and stopped by the addition of 0.1 ml of 2 M HCl. The incubation was carried out at 25°C for 1 min. An aliquot was evaporated to dryness and examined for incorporated radioactivity.

PEP carboxylase activity was determined according to Maruyama et al. (1966). The assay mixture (1 ml) contained 80 mM Tris-HCl (pH 7.8), 2 mM $MgCl_2$, 5 mM glutathion, 2 mM NADH, 14 unit ml^{-1} malate dehydrogenase (Boehringer Mannheim GmbH), 2 mM PEP, 10 mM [^{14}C]$NaHCO_3$ (0.5 mCi $mmol^{-1}$) and 0.02 ml of the supernatant. The reaction was started with the addition of PEP. The incubation was carried out at 25°C for 15 min and

stopped by the addition of 1 ml of 2 M HCl. An aliquot was dried and examined for incorporated radioactivity.

Other assays—Chlorophylls and carotenoids were determined photometrically (Strain et al. 1971, Metzner et al. 1965) for the extract from the leaf sections with 80% (v/v) acetone. Protein content was determined according to Lowry et al. (1951).

Special chemicals—[^{14}C]$NaHCO_3$ (61.1 mCi mmol^{-1}), L-[U-^{14}C]malic acid (160 mCi mmol^{-1}) and [1,5-^{14}C]citric acid were obtained from Commissariat a l'Énergie Atomique (CEA). Sodium [1-^{14}C]acetate (50.0 mCi mmol^{-1}) and [U-^{14}C]glycerol (11.8 mCi mmol^{-1}) were purchased from New England Nuclear.

Thiolactomycin was a generous gift from Chugai Pharmaceutical Co. Ltd., Tokyo.

Results

CO_2 fixation in etiolated leaves during greening of Avena *seedlings*—Fig. 1a shows the changes in CO_2 fixation rate in greening *Avena* leaves. There was some CO_2 fixation activity in the etiolated leaves independent of light. The activity in the light increased for the initial 2 h, then decreased to a small extent at 3 h, and again increased until 24 h. The activity in the dark remained unchanged throughout the greening. Since there was no synthesis of chlorophylls in the leaves during the initial 3 h (Fig. 1b), it is plausible to conclude that the CO_2 fixation at the beginning of greening proceeded by a path other than photosynthesis.

Fig. 2 shows changes in the main products fixed from [^{14}C]$NaHCO_3$ by greening *Avena* leaves under light. In etiolated leaves and 2 h-greened leaves, C_4-dicarboxylic acids such as malate, aspartate and glutamate, and citrate occupied more than 90% of the fixation product. After 3 h of greening these acids decreased while sucrose, triose-phosphate and alanine increased. These results indicate that CO_2 fixation in greening *Avena* leaves occurs by PEP carboxylation at earlier stages and by RuBP carboxylation at later stages. Fig. 3 shows changes in the activities

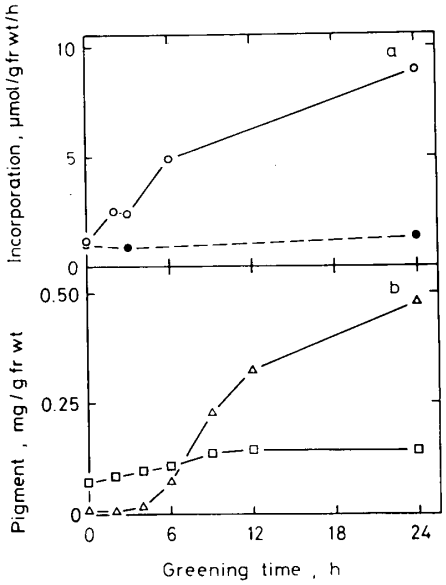

Fig. 1

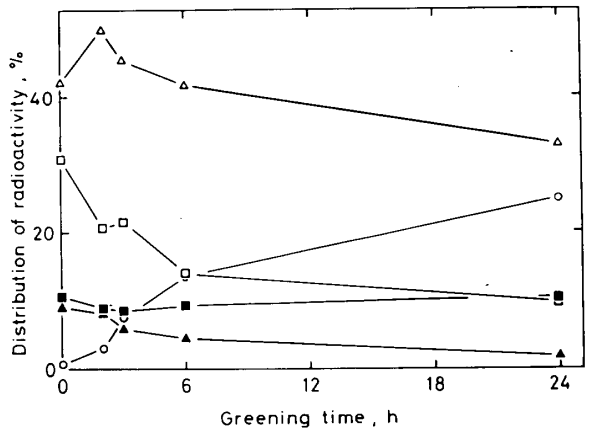

Fig. 2

Fig. 1 (a) Fixation of [^{14}C]$NaHCO_3$ by etiolated and greening *Avena* leaves in the light (-○-) and in the dark (--●--). (b) Chlorophyll (△) and carotenoid (□) content of etiolated and greening *Avena* leaves.

Fig. 2 Distribution of radioactivity of main products fixed from [^{14}C]$NaHCO_3$ by etiolated and greening *Avena* leaves. △, Malate; □, aspartate; ■, glutamate; ▲, citrate; ○, sucrose.

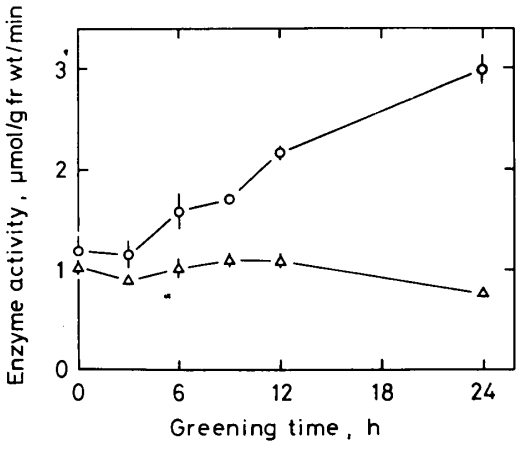

Fig. 3

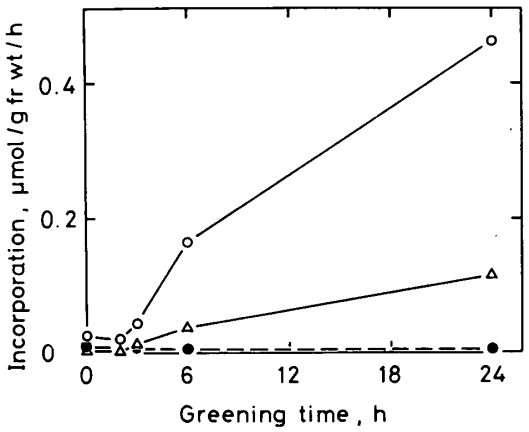

Fig. 4

Fig. 3 Levels of carboxylation enzymes in etiolated *Avena* leaves on illumination. The vertical lines represent the standard error. ○, RuBP carboxylase; △, PEP carboxylase.

Fig. 4 Incorporation of [^{14}C]$NaHCO_3$ into lipids and their fatty acid moieties by etiolated and greening *Avena* leaves. -○-, Lipids on illumination; --●--, lipids in darkness; -△-, fatty acid moiety of lipids on illumination.

of the two carboxylating enzymes, PEP carboxylase and RuBP carboxylase, during greening of etiolated leaves. The low level of PEP carboxylase activity was little changed throughout greening, whereas the some level of RuBP carboxylase activity was maintained during the initial 3 h of greening and thereafter increased continuously. Although the level of RuBP carboxylase was higher than that of PEP carboxylase at earlier stages of greening (the first 3 h), it was found that CO_2 was being fixed into the major products by PEP carboxylase. This is due to the absence of Calvin cycle activity, because the main products (more than 88%) of CO_2 fixation in the dark were malate, aspartate, glutamate and citrate even in 24 h-greened leaves (data not shown). On the other hand, at later stages of greening in which the Calvin cycle is fully operative, the products of CO_2 fixation are responsible for the increased activities of RuBP carboxylase.

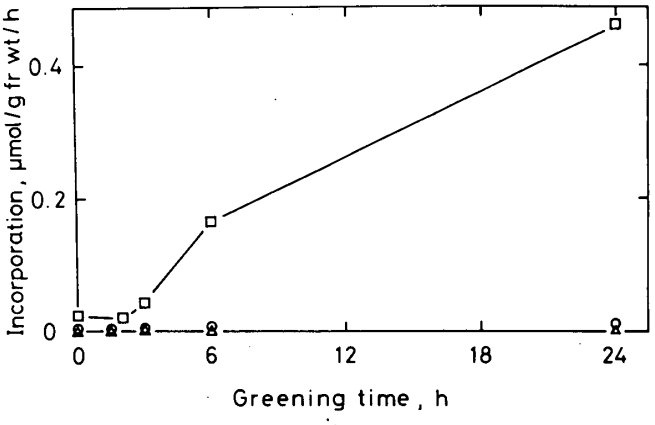

Fig. 5

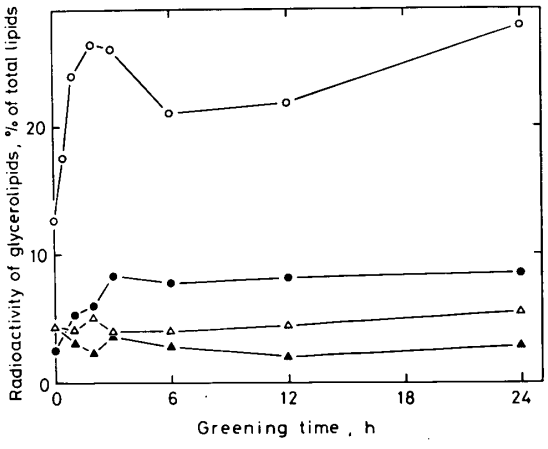

Fig. 6

Fig. 5 Incorporation of [^{14}C]malate (○), [^{14}C]citrate (△) or [^{14}C]$NaHCO_3$ (□) into lipids by etiolated and greening *Avena* leaves.

Fig. 6 Distribution of radilactivity of glycerolipids in lipids fixed from [^{14}C]$NaHCO_3$ by etiolated and greening *Avena* leaves. ○, PC; ●, PG; △, MGDG; ▲, DGDG.

Lipid synthesis from CO_2 during greening—The activity of lipid synthesis from CO_2 in the light was extremely low during the initial 3 h of illumination, but rapidly increased with greening (Fig. 4). The increase paralleled the development of photosynthesis in the leaves (Fig. 1a). In the dark no lipid synthesis from CO_2 was seen at any stage of greening. This suggests that the lipid synthesis from CO_2 was being derived from the photosynthetic path.

The possibility that the synthesis of lipids from CO_2 via malate and/or citrate was excluded by the absence of [^{14}C]malate or [^{14}C]citrate incorporation into lipids throughout greening (Fig. 5). Thus, it follows that de novo synthesis of lipids in greening leaves is being derived from photosynthetic products via the Calvin cycle.

Synthesis of lipid classes during greening—The synthetic rate of lipid classes from CO_2 in the light was shown in Fig. 6. The rate of the synthesis of both PC and PG increased during the initial 3 h, but increased little thereafter. There was no marked change in the rate of the synthesis of MGDG and DGDG during greening.

Development of fatty acid synthesizing system during greening—When the leaves were incubated with acetate, there were distinct activities of lipid synthesis in the etiolated and 3 h-greened leaves (Fig. 7), unlike the absence of lipid synthesis from CO_2 in these leaves (Fig. 4). These results

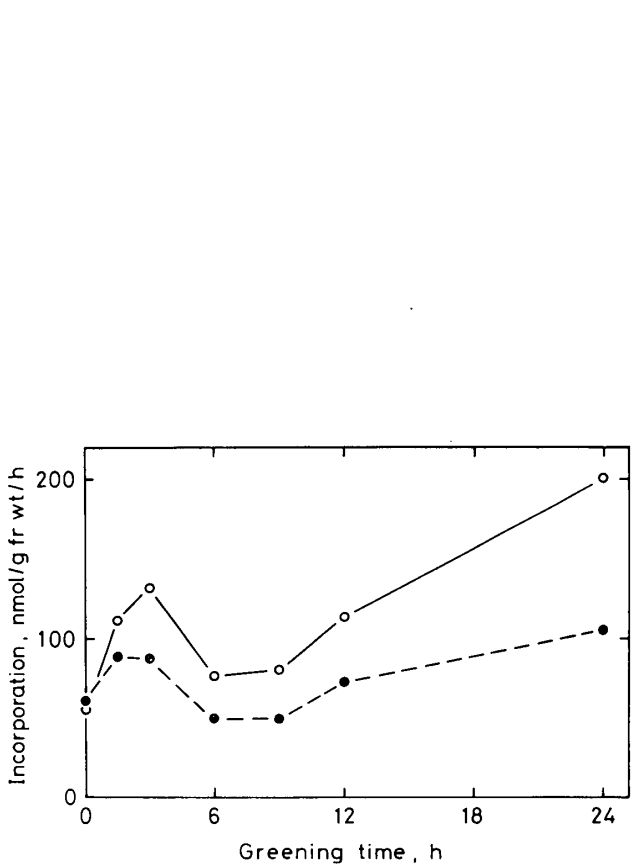

Fig. 7

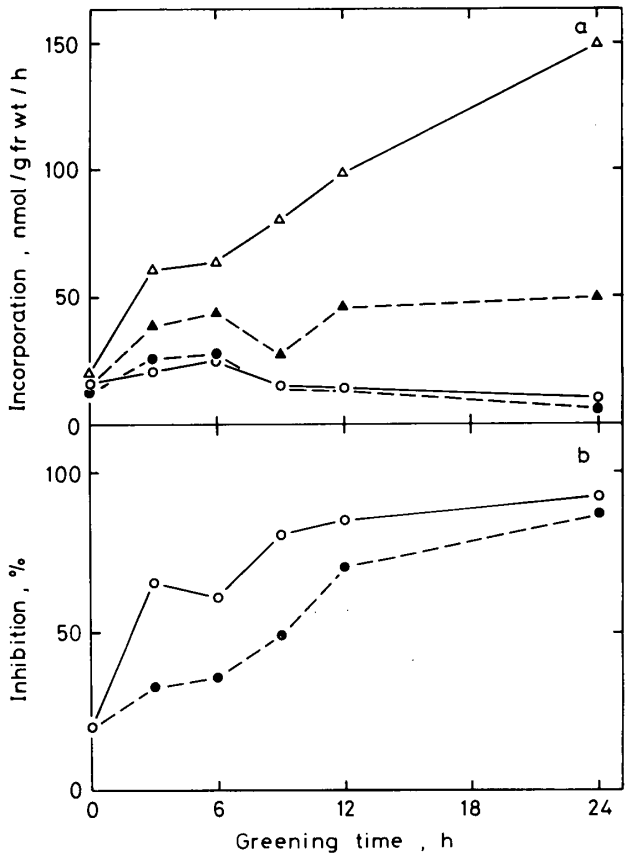

Fig. 8

Fig. 7 Incorporation of [^{14}C]acetate into lipids by etiolated and greening *Avena* leaves in the light (-○-) and in the dark (--●--).

Fig. 8 Effect of thiolactomycin on incorporation of [^{14}C]acetate into lipids by etiolated and greening *Avena* leaves. (a) Presence of thiolactomycin (4.08 μg ml^{-1}) in the light (○) and in the dark (●), absence of thiolactomycin in the light (△) and in the dark (▲). (b) Inhibition rate of lipid synthesis by thiolactomycin in the light (-○-) and in the dark (--●--).

Table 1 Incorporation of [^{14}C]$NaHCO_3$ into lipid intermediates by greening *Avena* leaves

Greening (h)	Total (nmol·g^{-1} fr wt·h^{-1})	Distribution (%)				
		Acyl-ACP	FA	Acyl-CoA	DG	Polar lipids
0	38	1.2	6.4	39.5	8.1	44.8
6	190	0.4	7.4	10.5	20.8	60.9
24	510	0.3	5.7	9.4	25.7	58.9

indicate that the metabolic path from acetate to lipids was previously operative in etiolated and 3 h-greened leaves before the path from CO_2 to acetate through the Calvin cycle was established. The synthetic activity from acetate in the light increased during the initial 3 h then decreased at 6 h, but afterwards increased again.

It is of interest to note that greening leaves possessed the capacity to synthesize fatty acids from acetate in the dark, although isolated plastids from these leaves did not (Ohnishi and Yamada 1980a). The activity in the dark ran at a lower rate than that in the light, but increased little after 12 h of greening. Fig. 8 shows the effect of thiolactomycin on fatty acid synthesis from acetate in greening *Avena* leaves. Thiolactomycin is a selective inhibitor of type II fatty acid synthetase which is located in the chloroplasts and plastids (Nishida et al. 1984). Incubation of the leaves with thiolactomycin inhibited the synthetic activity to some extent both in the light and dark (Fig. 8a). The inhibition rate was higher at later stages than at earlier stages of greening (Fig. 8b). The insensitivity of fatty acid synthesis to thiolactomycin at earlier stages suggests that the fatty acid synthesizing system in etioplasts and immature etiochloroplasts is different from that in mature chloroplasts.

Supply of carbon source to lipid synthesis—Polar lipids such as phospholipids and glycolipids are synthesized by the addition of a polar group to diacylglycerol moiety. These lipids are synthesized by the metabolic sequence: fatty acyl-ACP (the final product of fatty acid synthetase)→fatty acid→fatty acyl-CoA→PA (by the esterification with G3P)→DG→polar lipid (by the addition of polar group). Table 1 shows radioactivities of intermediates in these reactions, when [^{14}C]$NaHCO_3$ was incorporated into lipids by greening leaves. The accumulation of labeled DG increased with greening, followed by a decrease of labeled acyl-CoA, although the labels of acyl-ACP and fatty acids were kept at lower levels throughout greening. Radioactivity of PA fraction in polar lipids was virtually undetectable. These results suggest that the step supplying a polar group to diacylglycerol was a rate-limiting one at later stages of greening during which a steady synthesis of polar lipids is taking place (Fig. 6).

Incorporation of [^{14}C]glycerol into lipids increased with greening, but the accumulation of G3P did not (Table 2). This suggests a regulated supply of glycerol moiety to acyl moieties in lipid synthesis by greening leaves. This is supported by the distribution of radioactivity in the

Table 2 Incorporation of [U-^{14}C]glycerol by greening *Avena* leaves

Greening (h)	Total (nmol·g^{-1} fr wt·h^{-1})	Distribution (%)	
		G3P	lipids
0	113	31	9.3
6	423	36	11.5
12	432	33	17.4

Table 3 Radioactivity in lipid carbons synthesized from [^{14}C]$NaHCO_3$ by 24 h-greened *Avena* leaves

Lipid	Radioactivity (10^3 dpm C-number^{-1} [a])		
	Acyl	Glycerol	Head
PC	3.5	2.7	3.0
MGDG	0.44	0.54	2.1
DGDG	0.11	0.23	0.48

[a] Carbon number: Acyl moiety, 36; Glycerol moiety, 3; Head moiety, 5 (PC), 6 (MGDG), 12 (DGDG).

lipid molecules synthesized. When [^{14}C]$NaHCO_3$ was incorporated into phospholipids and glycolipids in 24 h-greened leaves, the radioactivity ratio on carbon atom basis in acyl, glycerol and polar groups were 1 : 1 : 1, for PC, 1 : 1 : 4 for MGDG and 1 : 2 : 4 for DGDG (Table 3). An equal distribution of radioactivity in PC molecule suggests the regulated supply of acyl, glycerol and choline moieties to the synthesis of PC. On the other hand, a denser distribution of ^{14}C in galactose moiety than that of the residual moiety in both MGDG and DGDG points to the fact that galactose moiety was synthesized more actively than acyl and glycerol moieties. This seems to suggest that in leaves the major flow of carbon metabolism from the Calvin cycle is directed to the synthesis of carbohydrate and that the minor flow is to the synthesis of lipid skeleton, acyl and glycerol moiety.

Discussion

Photosynthetic carbon fixation in greening *Avena* leaves was established 3 h after etioplasts began to develop into chloroplasts (Fig. 1a). After this stage, the carbon source of membrane lipids in greening leaves, especially in proliferating thylakoid membranes, was exclusively supplied from photosynthetically fixed CO_2 (Fig. 4). Prior to this, some CO_2 fixation by PEP carboxylase occurred, but its products, such as malate and citrate, were not utilized as precursors for fatty acid synthesis in *Avena* etioplasts and etiochloroplasts (Fig. 5), although fatty acid synthesis from acetate was already operative (Fig. 7). The activity of fatty acid synthesis in greening *Avena* leaves showed two phase curves, one at the earlier stages and one at later stages, with the activity at the earlier stages being less sensitive to thiolactomycin (Fig. 8b). This distinct property of fatty acid synthetase in the earlier stages of greening may offer a due to solving the mechanism behind the supply of lipids to proliferating cellular membranes during the formation of etiolated leaves at which stage plastids have not yet been differentiated in the cells, in contrast to fatty acid synthetase in foliage cells which is restricted to the plastids.

The metabolic flow of photosynthetically fixed CO_2 to fatty acid synthesis was about one-hundredth as much as that of photosynthetically fixed CO_2 to carbohydrate synthesis in leaves (Fig. 1a, Fig. 4). Therefore, an excess supply of G3P to acyl-CoA is to be expected in the formation of lipid molecule, because G3P is easily synthesized by a one-step reaction from dihydroxyacetonephosphate, an intermediate in the Calvin cycle, whereas the synthesis of acyl-CoA from dihydroxyacetonephosphate requires many steps. The same is true for the synthesis of the polar head group of lipids. However, the present study indicates that the supply of the constituents in lipid molecule architecture is well regulated so that the carbon labels are equally distributed among lipid constituents, glycerol, fatty acyl and polar head moieties (Table 3). On the other hand, the denser distribution of ^{14}C in galactose moiety than in the other car-

bons of MGDG and DGDG suggests that the galactose moiety is supplied by a metabolic flow differently regulated from that for the synthesis of glycerol and fatty acyl moieties. In this labeling experiment a lower label of diacylglycerol moiety in MGDG than in PC would support our earlier proposal that MGDG is synthesized from PC via intermediates (Ohnishi and Yamada 1980b).

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