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THE EFFECT OF LIGHT INTENSITY ON GALACTOLIPID SYNTHESIS IN *VICIA FABA* LEAVES

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Key Word Index—*Vicia faba*; Leguminosae; broad bean; lipid metabo ism; galactolipid; light.

Abstract—The effect of light intensity upon galactolipid synthesis in *Vicia faba* leaf tissue was studied at two CO_2 concentrations, 0.03 and 1%. The rates of galactolipid synthesis were estimated by determining the amount of radioactivity in each of the two galactoses of digalactosyl diacylglycerol (DGDG) and the single galactose of monogalactosyl diacylglycerol (MGDG), a technique based upon the accepted pathway for galactolipid synthesis in which galactosylation is the terminal step in biosynthesis. The results suggest that the rates of MGDG and DGDG synthesis were similar under all conditions and that galactolipid synthesis was not directly affected by light intensity. The quantity of radioactivity incorporated into the galactoses of individual molecular species of MGDG and DGDG were similar under the light conditions used.

INTRODUCTION

Monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) are the major glycerolipids of green tissue and, along with phosphatidyl glycerol (PG) and sulphoquinovosyl diacylglycerol (SL), are associated particularly with chloroplast thylakoid membranes. The effects of light upon the lipid and fatty acid composition of lipids during the greening of etiolated plant leaves have been investigated extensively [1–5]. However, attempts to show direct light control of galactolipid biosynthesis, either by its effect upon the availability of precursors or of ATP and $NADPH_2$, have not been conclusive [4, 6, 7]. It is generally accepted that MGDG is formed by the addition of galactose to a diacylglycerol and that DGDG is formed by the addition of galactose to MGDG, as proposed by Ferrari and Benson [8].

We have previously shown that the 3 moieties of the galactolipid molecule—galactose, glycerol and fatty acid—are labelled at different rates after feeding $^{14}CO_2$ [9]. The two galactoses of DGDG also show different labelling kinetics [10]. The amount of label in the outer galactose of DGDG (DGII) represents newly formed DGDG while the inner galactose (DGI) labelling originates from MGDG which has been converted to DGDG [8, 10]. As a consequence, it is not possible to determine rates of galactolipid synthesis after ^{14}C feeding by analysing the increase in radioactivity of the total lipid. Our previous results suggest that the amount of label in the galactose moieties would give an estimate of galactosyl transferase activity, which could be used to calculate galactolipid formation since galactosylation is the final metabolic step in their synthesis. Therefore, a method is possible for determining the rates of MGDG and DGDG synthesis. MGDG synthesis may be calculated by the addition of the amount of radioactivity found in the galactose of MGDG plus the amount of radioactivity found in DGI while DGDG synthesis may be calculated from the labelling of DGII alone.

This report demonstrates the use of this technique in an investigation into the effect of light intensity upon galactolipid biosynthesis, with a view to the determination of the type of control that light exerts.

RESULTS

The experiments reported here were designed to determine the effects of light intensity upon galactolipid synthesis in mature *Vicia faba* leaf tissue at both a normal concentration of 0.03% CO_2 and at an elevated level of 1% CO_2. The use of the two concentrations of CO_2 allows a comparison between a system in which CO_2 becomes limiting at high light intensity, with one in which light saturation does not occur at light intensities up to 19 klx. In the former case, any increase in lipid synthesis at high light intensity could be attributed to the production of high energy intermediates (ATP and/or $NADPH_2$) but not lipid precursors, while in the latter case, an increase could also be due to an increase in lipid precursors.

The rates of photosynthesis of the leaf discs at each light intensity were found by measuring the ^{14}C incorporation of a random set of 8 leaf discs after each feeding (Fig. 1). The light saturation point (the point at which an increase in the light intensity produced no effect upon the photoassimilation rate) was *ca* 11 klx. In direct contrast, the rate of photosynthesis increased steadily for 1% CO_2 at increasing light intensities and light saturation of photosynthesis was not observed under these conditions.

In Fig. 1, the rates of photoassimilation of ^{14}C in 0.03% and 1% CO_2 are compared with the rates of ^{14}C incorporation into the galactoses which indicate the rate of MGDG synthesis (the galactose of MGDG plus DGI) and DGDG synthesis (DGII) at different light intensities.

The quantity of radioactivity found in the galactoses

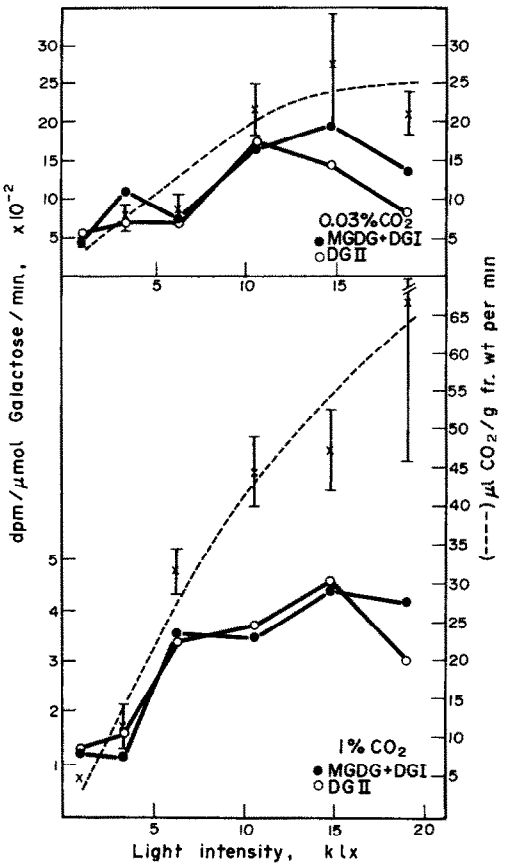

Fig. 1. Rate of ^{14}C incorporation into the galactoses representing the rate of MGDG synthesis (MGDG + DGI) and the rate of DGDG synthesis (DGII) at 0.03% CO_2 and 1% CO_2 compared with the rate of photoassimilation at different light intensities and two concentrations of CO_2.

Table 1. Ratio of the amount of radioactivity found in the galactoses associated with MGDG and DGDG synthesis at different light intensities and two concentrations of CO_2

Light intensity (klx)	MGDG + DGI* / DGII	
	0.03% CO_2	1% CO_2
1.1	0.8	0.9
3.4	1.6	0.7
6.4	1.1	1.0
10.7	1.0	0.9
15.0	1.4	1.0
19.3	1.6	1.4
Mean	1.2 (±0.3)	1.0 (±0.2)

* Ratio of the sum of the amount of radioactivity associated with the galactose of MGDG and the inner galactose of DGDG (MGDG + DGI) representing the rate of MGDG synthesis to the amount of radioactivity found in the outer galactose of DGDG (DGII), which represents the rate of DGDG synthesis.

in the 1% CO_2 experiment was much lower than that found in the same fractions at 0.03% CO_2. This was due to the dilution of the same initial quantity of radioactivity fed to the leaf discs at 1% CO_2 by the added $^{14}CO_2$. In Fig. 1, the scale for the rate of ^{14}C incorporation into the galactoses at 1% CO_2 has been expanded by a factor equal to the differences in the sp. act. of the two CO_2 concentrations used. The sp. act. of the two galactoses cannot, therefore, be compared directly but the trends may be used as an indication of the rates of synthesis after this correction for the sp. act. of fed $^{14}CO_2$ has been made.

Despite the differences in the trends for photoassimilation at the two concentrations of CO_2, the rates of MGDG and DGDG synthesis show the same type of saturation curve in both experiments. This may be explained with reference to the pool of galactolipid precursor (presumably UDP-galactose), the sp. act. of which directly affects the level of radioactivity in the galactose of the galactolipid molecule. At low light intensity, the sp. act. of this pool is probably increasing at increasing light intensities for both 0.03% and 1% CO_2 during the time of incubation. The time of incubation was insufficient for a steady state to be achieved between the $^{14}CO_2$ and the UDP-galactose pool. At higher light intensities, a steady state may be reached more rapidly within the incubation time. The apparent increase in sp. act. of the galactoses at higher light intensities is likely due to the rate at which the steady state is attained. This would be dependent upon the rate of CO_2 fixation until the steady state is reached.

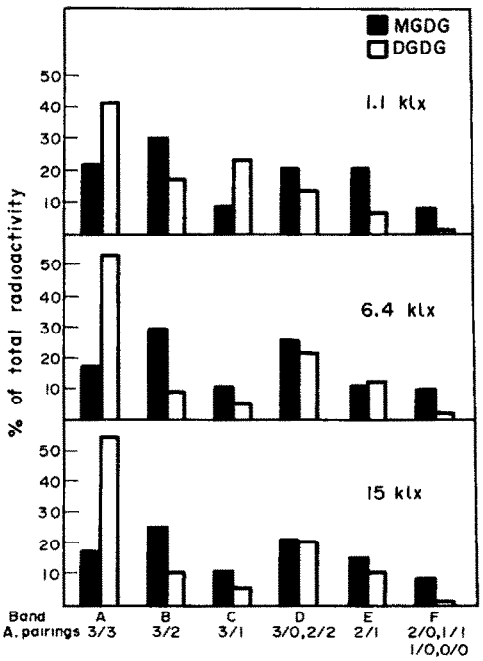

Fig. 2. Distribution of radioactivity among the molecular species of MGDG and DGDG (1% CO_2) under different light intensities. Band 'A' contains the molecular species with 6 double bonds (Fatty acid pairing: 3/3), band 'B' contains the molecular species with 5 double bonds (Fatty acid pairing: 3/2), etc.

Only after a steady state is established can a true measure of lipid synthesis be made. In these experiments, it appears that the galactose labelling levelled off at a lower light intensity in 1% CO_2 than in 0.03% CO_2 because of a more rapid attainment of the steady state due to the higher rate of CO_2 fixation.

In the 1% CO_2 experiment, the rate of incorporation of radioactivity into the galactose did not increase with increasing the light intensity above *ca* 6 klx. This suggests that neither light intensity nor photosynthesis has a direct effect on galactolipid synthesis. In the 0.03% CO_2 experiment, the radioactivity of the galactose levelled off at a higher light intensity but at a similar level (after taking differences in the sp. act. of the fed $^{14}CO_2$ into account) as that of the 1% CO_2 experiment. These results appear to confirm that the rate of galactolipid synthesis is not determined by light intensity or CO_2 fixation in short term experiments.

The rate of assimilation of ^{14}C into DGII (DGDG synthesis) was similar to that into MGDG galactoses (MGDG + DGI) at both concentrations of CO_2 and all light intensities. A quantitative estimate of the relative rates of synthesis of the two galactolipids was made from the calculation of the ratios of the sp. act. MGDG + DGI/DGII (Table 1). The ratio was 1 ($\pm$0.2) for 1% CO_2 and 1.2 ($\pm$0.3) for 0.03% CO_2. No significant effect upon the relative rates of synthesis of MGDG or DGDG was observed at increasing light intensities or different CO_2 concentrations.

In a previous study [11], it was shown that the labelling of molecular species of MGDG gave an anomalous result. Despite the fact that the major molecule species of MGDG is 18:3/18:3, the majority of the label was found in the 18:2/18:3 species. This is confirmed in this study and is shown not to result from differences in light intensity used during $^{14}CO_2$ feeding (Fig. 2). Clearly this anomaly is not due to differences in light intensity over the short term used in feeding experiments. The results also indicate that the labelling pattern of the molecular species of MGDG and DGDG differ and that this is consistent at different light intensities.

DISCUSSION

The observed incorporation of radioactivity into galactoses of *Vicia faba* was similar despite the differences in photoassimilation at the two concentrations of CO_2 and different light intensities. A light saturation curve for photoassimilation by leaf discs was observed at 0.03% CO_2, reflecting the limitation upon the capacity of the carboxylating enzyme at 11 klx whereas photoassimilation at 1% CO_2 increased almost linearly with light intensity. The levelling off of ^{14}C incorporation into the galactoses of the galactolipid was interpreted to be due to the equilibration of radioactivity of the lipid precursor pool with fed $^{14}CO_2$.

Our results suggest that the rate of lipid synthesis may have been the same at all light intensities in both concentrations of CO_2 and that the initial increase in radioactivity was due to differences in the rates of equilibration of the lipid precursors with the fed $^{14}CO_2$.

The data seem to eliminate the possibility that light controls lipid synthesis by controlling the availability of ATP and $NADPH_2$ [6]. They indicate instead, that the reactions involving galactose formation and the availability of lipid precursors may be controlling steps although this was not demonstrated in these experiments.

EXPERIMENTAL

$^{14}CO_2$ feeding. Mature leaves were harvested from 21-day-old broad bean (*V. faba* cv Giant Windsor) plants grown in growth cabinets at 21 $\pm$ 1° and 21 klx (16 hr light: 8 hr dark). Leaf discs (64) (18 mm) were cut from the leaves and positioned on damp cheese-cloth for equilibration (15 min) at the light intensity used for the expt (indicated in the text). The leaf discs were allowed to assimilate CO_2 (0.03 or 1%) containing 300 μCi $^{14}CO_2$ for 10 min (0.03%) or 15 min (1%) and the feeding was followed by a 'chase' of 10 min. The normal concn of CO_2 (0.03%) was maintained by passing a large vol. of air containing 300 μCi $^{14}CO_2$ through the feeding chamber (3 l. capacity) at a rate of 1–1.5 l./min.

Derivatization of MGDG and DGDG. Lipids were extracted from the leaf tissue according to the method of ref. [12]. MGDG and DGDG were separated by TLC of the green total lipid extract on Si gel G using Me_2CO–C_6H_6–H_2O (91:30:8) [13]. The galactolipids were deacylated with NaOMe and derivatives of the galactoses of MGDG and DGDG were prepared by methylation with methyl sulphinyl Na anion in DMSO and excess CH_3I [10]. A known amount of cold MGDG or DGDG was added to the radioactive samples before derivatization. Methylation and methanolysis of DGDG produced 2 galactoses differing by 1 Me group (DGII. Me-2,3,4,6-tetra-*O*-Me galactoside, and DGI, Me-2,3,4-tri-*O*-Me galactoside). To improve separation of the 2 galactoses by GLC, the samples were dried under N_2 at 37°, redissolved in 100 μl Py and 50 μl Ac_2O and heated at 100° for 30 min to produce Me-2,3,4-tri-*O*-Me-6-mono-*O*-acetyl galactoside from DGI.

GLC. The following conditions were used with a FID detector: derivatized galactoses were analysed with temp. programming (initial temp. 110°, initial hold 4 min, rate 5° min, final temp. 200°) on a column (1200 × 4 mm) packed with 3% JXR on Chromosorb Q. The N_2 flow rate was 85 ml/min. The derivatized galactose fractions of MGDG and DGDG were collected for liquid scintillation counting (LSC) during GLC according to ref. [14]. Me pentadecanoate was used as the internal standard in all samples.

LSC. A random set of 8 leaf discs were analysed by LSC after solubilization with 0.5 ml Protosol (New England Nuclear) and heating *ca* 18 hr at 37°. The toluene based scintillation fluid contained PPO (6 g/l.) and POPOP (300 mg/l.). The galactoses from fraction collection during GLC were eluted from the glass beads with scintillation fluid directly into the vial for analysis.

Analysis of molecular species. MGDG and DGDG (obtained from the green total lipid extract as described above) were separated into their molecular species by TLC on Si gel G (40 g dissolved in 100 ml H_2O containing 10 g of dissolved $AgNO_3$) using $CHCl_3$–MeOH–H_2O (65:25:4 for MGDG and 60:30:4 for DGDG) [4, 11]. They were eluted from the gel with $CHCl_3$–MeOH–H_2O–NH_4OH (23:13:3 plus 0.5 ml NH_4OH containing 28–29% NH_3) [15]; the washings were placed in scintillation vials and dried under N_2 at 37° prior to LSC analysis using the scintillation fluid described.

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