

A Possible Mechanism for a Light-Driven Regulation of the Fatty Acid Composition in Galactolipids of Chloroplasts

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Acylation Mechanism, Galactolipid Synthesis

The occurrence of acylgalactosylglycerol (AGG) in spinach chloroplasts is proposed. In lipid separations of whole spinach leaves this fraction is hidden by variable amounts of extraplastidary steryl glucoside (SG) which depended on the physiological state of the leaf material. Beside the phospholipid fraction, this lipid mixture with unique chromatographic behavior, recently termed GL, exhibited the highest specific activity in the lipid extract of infiltrated leaf sections of spinach after short periods of illumination (2 min) under ^{14}C -fixing conditions. The main source of label was found in the fatty acid residues of the lipids described. The decrease of specific activity in GL after a cold chase was accompanied by an increasing ^{14}C -incorporation into the fatty acid moieties of the MGDG- as well as the phospholipid fraction. This effect was significantly reduced if the ^{14}C -pulse was followed by a dark period. This incorporation behavior suggests, that AGG functions as an intermediary acyl acceptor in the light driven fatty acid transfer between the lipids described. SG-synthesis, on the contrary, is independent of additional illumination and seems to be localized outside the chloroplast. The last notion was derived from experiments involving the incorporation of $[\text{UDP-}^{14}\text{C}]$ glucose into SG by spinach leaf homogenates. With regard to the increasing level of trienoic fatty acids in AGG and MGDG during the regeneration of dark pretreated spinach leaves in the light, the data are interpreted in terms of a light regulated acylation of AGG with specific unsaturated fatty acids.

Introduction

One of the key problems in the metabolism of galactolipids in higher plants and algae is to understand the accumulation of higher unsaturated fatty acids in this lipid class.

The following hypotheses, recently reviewed by Douce and Joyard [1], may represent the present state of information about the origin of polyunsaturated fatty acids in chloroplast galactolipids:

1) The desaturation of fatty acids occurs *after* the formation of the galactolipid molecule [2].

2) The desaturation occurs *before* the formation of the galactolipid molecule [3]. a) All desaturases involved are confined to the chloroplast. b) The conversion of oleic to linoleic acid is maximal in the microsomes, whereas the desaturation of linoleic to linolenic acid is highest in chloroplast membranes [4].

3) Phosphatidyl choline acts as a carrier molecule in the desaturation reaction [5].

4) Desaturation is attained by a deacylation-reacylation mechanism of galactolipid molecules [6].

The last mentioned hypothesis was derived from *in vitro*-experiments involving the enzymatic conversion of synthetically prepared lyso-MGDG to MGDG with leaf homogenates as well as acetone powder of spinach chloroplasts in the presence of CoA, ATP and *polyunsaturated fatty acids* [6, 7]. It should be mentioned that as yet the intermediate formation of lyso-MGDG could not be demonstrated, either *in vivo* or by galactolipase hydrolysis. This fact suggests that either the acylation or the deacylation of this compound prevents its accumulation and detection. Galactolipase catalyzes the gradual deacylation of galactolipids [8], as shown for example with MGDG:



Abbreviations: AGD, acylgalactosyl diglyceride; AGG, acylgalactosylglycerol; ASG, acylated steryl glucoside; CoA, coenzyme A; DGDG, digalactosyl diglyceride; GL in chloroplasts, AGG; GL in leaves, lipid mixture of SG and AGG; lyso-MGDG = MGMG, monogalactosyl monoglyceride; MGDG, monogalactosyl diglyceride; PL, total phospholipid fraction; SG, steryl glucoside; SL, sulphoquinovosyl diglyceride; UDP, uridine diphosphate.

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Lipid separations of spinach leaf extracts suggest the occurrence of a lipid with the same stoichiometry as lyso-MGDG in a glycolipid mixture with SG, showing unique chromatographic behavior. This lipid mixture, recently termed GL, with slightly more polar character than MGDG, exhibited ^{14}C -incorporation kinetics similar to a precursor in MGDG-synthesis [9, 10]. This paper is concerned with the possi-



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ble importance of the substance, which we designate as AGG, in the light dependent regulation of the fatty acid composition of galactolipids in chloroplast membranes.

Materials and Methods

^{14}C -incorporation into infiltrated leaf sections and their lipid extraction and analysis was carried out according to Heise and Krapf [10]. ^{14}C -incorporation into isolated chloroplasts has been described previously by Heise [11]. The incubation of spinach leaf homogenates with $[\text{UDP-}^{14}\text{C}]\text{glucose}$ was carried out by illuminating the same suspension (0.14 mg Chl/ml) for various amounts of time with white light ($2 \times 10^5 \text{ ergs} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$) in a water bath at 20°C under mechanical shaking. The incubation medium contained 1.0 ml leaf homogenate (0.14 mg Chl/ml) and 0.1 ml ($\cong 2.8 \text{ nmol}$) $[\text{UDP-}^{14}\text{C}]\text{glucose}$ solution ($\cong 0.83 \mu\text{Ci}$). Spinach leaf homogenate was obtained by fast homogenisation of leaves with Jensen-Bassham medium (A) and filtrating the slurry through four layers of cheese cloth. The entire procedure including the estimation of chlorophyll concentration lasted no more than five minutes. After incubation, the samples were centrifuged for five minutes at $1000 \times g$ to separate chloroplasts. Sediment and supernatant were extracted according to Bligh and Dyer [12]. Separation and analysis of the lipid extract was described by Heise and Jacobi [13], sterol derivatives were estimated according to Eichenberger and Grob [14]. AGG was synthesized by incubation of $1 \mu\text{mol}$ AGD with 3 mg of pancreatic lipase at 37°C for 1 h. AGD was isolated according to Heinz *et al.* [15]. Immediately after enzymatic hydrolysis, lipids were extracted according to Bligh and

Dyer [12]. An as yet unidentified lipid of the hydrolysate showed the same chromatographic behavior as SG. Because of its stoichiometry it was preliminarily designated as AGG.

Results and Discussion

After short illumination periods (2 min) under ^{14}C -fixing conditions the main source of label in lipid extracts of infiltrated spinach leaf sections was found in the fatty acid residues of phospholipids and of a glycolipid fraction with slightly more polar character than MGDG. This fraction was previously termed GL [9, 10]. The light dependent decrease of label in GL in favor of increasing ^{14}C -incorporation into MGDG and phospholipids during the following cold chase is represented in Fig. 1 by plotting the difference of the kinetics between light- and dark chase. This incorporation behavior suggests, GL to function as an intermediary acyl acceptor in the light stimulated fatty acid transfer between the lipids described. As recent analyses of its hydrolysate indicate, GL in leaf extracts contains sterol, glucose, galactose, glycerol and fatty acids in changing molar ratios dependent on the physiological state of the extracted leaf material. The mean value of the molar ratios (sterol : sugar : glycerol : fatty acids = 1 : 2.5 : 1.5 : 1.5) and the chromatographic behavior of this fraction points to a mixture of lyso-MGDG with SG. But under the separation conditions described, lyso-MGDG was slightly more polar than SG. Identical chromatographic behavior as SG after TLC (solvent system: $\text{CHCl}_3/\text{CH}_3\text{OH}$ 85 : 15 v/v) and a requisite molar ratio showed on the contrary an as yet unidentified lipid fraction (R_f 0.21) resulting from enzymatic hydrolysis of AGD. This was preliminarily designated as AGG.

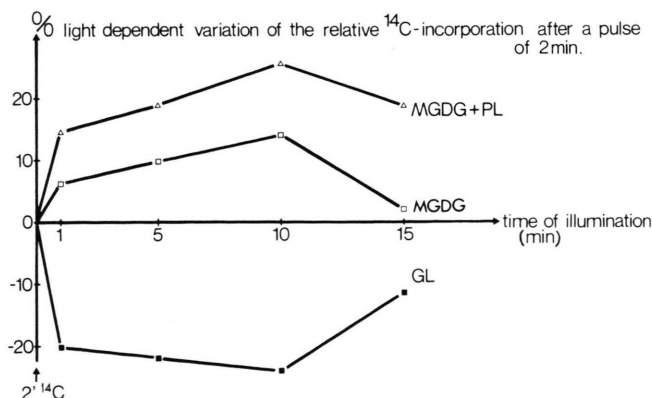
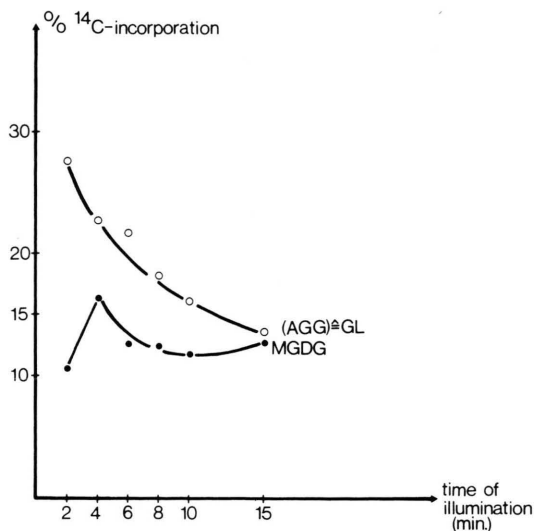


Fig. 1. Relative variation of ^{14}C -incorporation in the MGDG ($-\square-\square-$) or the combined MGDG- and phospholipid fraction ($-\triangle-\triangle-$) and the GL-fraction ($-\blacksquare-\blacksquare-$) of spinach leaves after a ^{14}C -pulse of 2 min in the light at pH 7.8. This figure shows the difference in kinetics between light- and dark chase. The ^{14}C -incorporation into the total lipid fraction was taken as 100%. The ^{14}C -label after the pulse of 2 min was arbitrarily set as starting point (0) of the incorporation kinetics. The data are mean values of three experiments.



Only after separation of the leaf homogenate we could show that AGG had to be assigned to the chloroplast while SG obviously was located outside. After sedimentation of chloroplasts in leaf homogenates differences in the lipid composition of the pellet and of the supernatant (Table I) point to the separation of GL into a plastidary (AGG) and an extraplastidary lipid portion (SG) under our conditions. The occurrence of two lipids with identical chromatographic properties but different comparti-

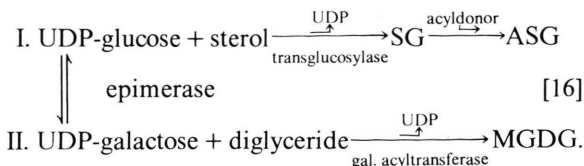
Table I. Lipid composition of a spinach leaf homogenate (A) and the approximative association of the lipids with the plastidary (B) and extraplastidary (C) compartment by sedimentation of the chloroplasts at $1000 \times g$ for 5 min and subsequent fat analysis of the pellet and the supernatant.

Lipids	Lipid concentration [μmol lipid/mg Chl.]		
	A.	B.	C.
MGDG	1.31	1.11	0.20
AGG	0.20	0.16	0.04
SG	0.18	0.03	0.15
MGMG	0.31	0.18	0.13
DGDG	0.59	0.51	0.08
SL	0.29	0.20	0.09
PL	0.89	0.25	0.64
	3.77	2.44	1.33

But the linear decrease of the relatively high source of label in GL (AGG) after short incubation periods (2 min) under photosynthetic conditions was not accompanied by a corresponding increase of ^{14}C -incorporation into MGDG, as was observed in leaf sections. The reduction of ^{14}C -incorporation capacity in polar lipids of isolated chloroplasts with increasing illumination periods was paralleled by an ^{14}C -accumulation in the neutral lipid fraction [11] and a decreasing CO_2 -fixation capacity of the chloroplasts. This effect suggests an inhibited synthesis of polar lipids by the reduction of intact chloroplasts.

Fast, light dependent reactions in lipid biosynthesis should be expected in chloroplast lipids first. Considering the above mentioned compartmentation (Table I), the light stimulated precursor function in MGDG-synthesis should therefore be attributed to AGG only. In view of the fact that in normal spinach leaves GL was mainly labelled in its fatty acid residues [9], the inverse incorporation behavior of GL and MGDG can be interpreted as acylation of AGG to MGDG and subsequent cleavage of the sugar bound fatty acid. A similar mechanism was already postulated for lyso-MGDG in "in vitro"-experiments [6] and led to an explanation for the desaturation of galactolipid molecules by means of a deacylation-reacylation mechanism.

As the following reaction sequence shows, UDP-glucose is a common precursor in SG- and galactolipid synthesis:



With the exception of the galactosylation of diglycerides – this reaction is localized in the chloroplast envelope [17] – the above reactions occur outside the chloroplast.

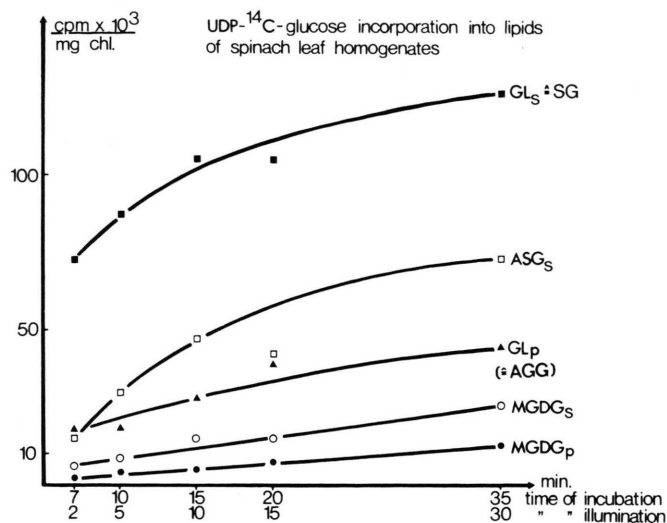


Fig. 3. ^{14}C -labelling kinetics from $[\text{UDP-}^{14}\text{C}]$ glucose into the glycolipids of spinach leaf homogenates in relation to the time of illumination. The distribution of label is followed between pellet (subscript P) and supernatant (subscript S) after sedimentation of chloroplasts at $1000\times g$. Particulars of the experiments are given in Methods. The data are averages of at least three independent experiments.

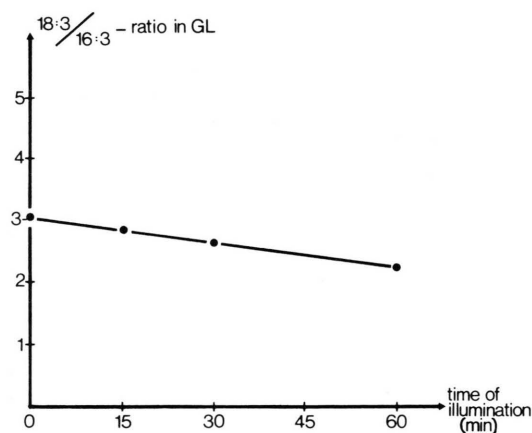


Fig. 4. Changes of the $\text{C}_{18:3}/\text{C}_{16:3}$ -ratio in the fatty acid pattern of GL of dark pretreated spinach leaves (4–5 days) in relation to the time of light regeneration (illumination). Because of the significant diurnal changes of the above fatty acid-ratio, the values of only one preparation with the same physiological age of the leaves was taken for this kinetics.

If spinach leaf homogenates were incubated with $[\text{UDP-}^{14}\text{C}]$ glucose and chloroplasts were separated immediately afterwards by sedimentation, the above biosynthetic pathways proved reasonable (Table II, Fig. 3). The main source of label of the lipid fraction was found in SG and ASG of the supernatant. This ^{14}C -incorporation into SG was independent of additional illumination (Table II). In contrast, the relative small but significant label of AGG and MGDG was apparently stimulated by illumination and by increasing chlorophyll concentration of the leaf homogenate (Table II). The latter was taken as criterion for an increasing integrity of its chloroplasts, being an important prerequisite for galactolipid synthesis. Summarizing the above results, the “*in vivo*”-occurrence of AGG in photosynthetic tissues with fatty acid patterns similar to that of MGDG [9] is shown. The present state of information gives no

Table II. Relative incorporation of $[\text{glucose-}^{14}\text{C}]\text{-UDP}$ into the lipids of spinach leaf homogenates. A. with 0.13 mg Chl/ml; B. with 0.29 mg Chl/ml in the light (l) ($2\times 10^5 \text{ ergs}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}$) and in the dark (d). 1.1 ml homogenate was incubated under mechanical shaking for 15 min at 20°C with 4.2 nmol ($=0.83 \mu\text{Ci}$) $[\text{glucose-}^{14}\text{C}]\text{-UDP}$ and centrifuged afterwards for 5 min at $1000\times g$ to separate chloroplasts.

			% incorporation of the total lipid fraction in						
			ASG	MGDG	AGG	SG	MGMG	DGDG	PL
A.		plastidial	4.2	3.3	17.0	—	0.5	0.6	0.3
		extraplastidial	18.4	6.5	—	46.0	1.3	1.4	0.5
B.	(l)	plastidial	—	5.2	23.3	—	1.3	2.0	0.9
		extraplastidial	2.5	12.5	—	47.4	1.3	2.8	0.8
	(d)	plastidial	—	3.9	17.9	—	1.1	1.7	0.7
		extraplastidial	3.1	12.8	—	51.6	2.9	3.2	1.1

evidence, whether it is synthesized by lipolytic deacylation of AGD or by galactosylation of the monoglyceride pool and subsequent acylation of the sugar residue in the chloroplast envelope. But the incorporation behavior of this compound suggests a light driven conversion of AGG to MGDG in spinach leaves. The acylation of AGG offers a mechanism to explain the origin of the high levels of polyunsaturated fatty acids in the galactolipids of chloroplast membranes.

The increasing level of trienoic fatty acids, especially that of hexadecatrienoic acid, in AGG during regeneration of dark pretreated (for 4–5 days) spinach leaves in the light [10] may give an impression of the high “fatty acid-turnover” in this lipid. The particular importance of α -C_{16:3} in AGG during this light regeneration is indicated in Fig. 4, showing the decreasing level of α -C_{18:3} compared to α -C_{16:3} in the fatty acid pattern of AGG dependent on the time of illumination.

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