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Galactolipid Synthesis in *Vicia faba* Leaves

IV. SITE(S) OF FATTY ACID INCORPORATION INTO THE MAJOR GLYCEROLIPIDS[1]

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ABSTRACT

The fatty acids of the major glycerolipids of *Vicia faba* leaves were analyzed immediately following $^{14}CO_2$ feeding. The leaves were fractionated into chloroplast and cytoplasmic fractions and the location of radioactivity in the fatty acids determined. The results indicate that the major site of incorporation of fatty acids is in the phospholipids. Phosphatidylcholine contained the highest level of radioactivity in the cytoplasmic fraction, whereas phosphatidylglycerol contained radioactivity in both the chloroplast and cytoplasmic fractions. The galactolipids contained very little radioactivity in comparison, this radioactivity being confined to high speed centrifugal fractions believed to contain the envelopes of the chloroplast. Our results suggest that phosphatidylcholine is a major site of incorporation of fatty acids (mainly in oleic acid) in the cytoplasm, whereas phosphatidylglycerol is also a site of incorporation involving both oleic and palmitic acids, inside and outside the chloroplast.

In recent years the location of fatty acid synthesis and desaturation in leaves has been a topic of some controversy. Smirnov (4), Mudd and McManus (2), and Stumpf and James (5) have suggested that the chloroplast is a major site of synthesis of saturated fatty acids in leaves. Roughan (3), and Williams *et al.* (8) have indicated that PC[2] in higher plant leaves may be associated with fatty acid desaturation and that the fatty acids may be synthesized and desaturated prior to incorporation into the galactolipids in chloroplasts. Roughan (3) proposed that an exchange between unsaturated fatty acid in PC and the fatty acid of MGDG and DGDG may occur, resulting in an increase in the level of unsaturated fatty acids in galactolipids. Williams *et al.* (8) provided evidence that the precursor diglyceride of MGDG was already significantly desaturated and that fatty acids were probably derived from PC in the form of a diglyceride. Because of the low levels of PC in chloroplasts, these steps would presumably occur outside the chloroplast. Williams *et al.* (8) have also shown that PG is highly labeled shortly following $^{14}CO_2$ feeding to leaves and this lipid may also be involved in fatty acid synthesis and desaturation. PG is found in significant quantities both inside and outside the chloroplast.

The purpose of this investigation was to determine where the initial sites of incorporation of radioactivity into the fatty acids of the two phospholipids and the galactolipids occurred. In our previous paper (7) we reported our analyses of galactose labeling from $^{14}CO_2$ feeding in different centrifugal fractions of broad bean leaf galactolipids. The fatty acids were analyzed in the same experiments and the data from these experiments are reported here.

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[2] Abbreviations: PC: phosphatidylcholine; PG: phosphatidylglycerol; MGDG: monogalactosyl diglyceride; DGDG: digalactosyldiglyceride.

MATERIALS AND METHODS

The plant material, sampling, cell fractionation, and extraction of lipids have been described in the previous paper (7).

Fatty acids were analyzed after methanolysis in 1.5 N HCl in methylalcohol for 16 h at 80 C and extraction into hexane. Methyl pentadecanoate was used as an internal standard and the fatty acid methyl esters were separated on glass columns (1.2 m × 4 mm i.d.) packed with 10% EGSS-X (Chromosorb P) or 10% Silar-10 C (Chromosorb Q) run isothermally at 180 C. The effluent stream was divided and the fatty acid methyl esters were collected for radioactivity measurement according to the method of Watson and Williams (6).

RESULTS

The Chl and lipid contents, including fatty acid composition, of the leaves used in this study are contained in the previous paper (7). As discussed in that paper cell homogenates were separated into fractions containing predominantly broken and intact chloroplasts and into higher speed centrifugal fractions (10,000*g*, 40,000*g*, and 144,000*g*) containing at least half of the envelopes, and the bulk of mitochondria and microsomal membranes.

In Table I the results of the distribution of lipid and radioactivity in fatty acid are shown for the four major lipids of the leaves. The leaves were macerated and separated into fractions immediately following a 10-min $^{14}CO_2$ feeding and it is therefore possible to determine the initial sites of $^{14}CO_2$ incorporation into the fatty acids of the glycerolipids. At this time the majority of the radioactivity is found in the two phospholipids (PC and PG) with little activity in the galactolipids. The total radioactivity data confirm our earlier findings (8) that PC and PG are the major sites of initial incorporation of fatty acid into glycerolipids.

For MGDG, DGDG, and PC the greatest amount of radioactivity was found in the 10,000*g* and 40,000*g* fractions. Very little activity was found in these lipids from the chloroplast fractions. In contrast, approximately one-third of the activity in PG was found in the chloroplast fraction, with the remainder mainly in the 10,000*g* and 40,000*g* fractions. These data suggest that the chloroplast thylakoids are not the major site of fatty acid incorporation into the galactolipids but are a significant site of fatty acid incorporation into PG. Despite the fact that over 80% of the galactolipid was found in the two chloroplast fractions they contained less than 20% of the radioactivity. The major part of the radioactivity was incorporated into fractions presumed to contain envelope material and other cytoplasmic membranes.

The data for PC are consistent with the idea that the major site of fatty acid synthesis associated with PC is in the cytoplasm and not in the chloroplast. Although the total radioactivity accumulated in the chloroplast PC exceeded that of the two galactolipids,

Table I. Chlorophyll and lipid distribution and radioactivity of the fatty acids of the major glycerolipids, in cell fractions of Vicia faba leaves separated by sucrose gradients and high speed centrifugation.

The total pigment and lipid recovered in all fractions of the homogenate was: chlorophyll, 4.31 µmoles;MGDG, 3.67 µmoles; DGDG, 3.45 µmoles; PC, 1.28 µmoles; PG, 1.06 µmoles (see Table I, ref. 7). The radioactivity in each fraction was the sum of the radioactivities of the individual fatty acids in Table II.

Fraction	Chl	MGDG		DGDG		PC		PG	
		Lipid	Radio-activity	Lipid	Radio-activity	Lipid	Radio-activity	Lipid	Radio-activity
	%	%	% cpm[1]	%	% cpm	%	% cpm	%	% cpm
Broken chloroplast	87.9	74	12 193	76	8 42	10	4 571	66	29 1240
Intact chloroplast	6.1	8	7 110	6	10 50	7	1 95	5	6 261
10,000xg	6.0	14	44 701	8	40 203	47	65 9671	13	33 1434
40,000xg	tr[2]	3	29 468	6	26 132	22	35 3708	8	21 894
144,000xg	tr	2	8 135	4	17 85	14	5 713	8	11 491
Total radioactivity (cpm)			1607		512		14758		4320

[1] after subtraction of background counts
[2] less than 0.05%

Table II. Radioactivity in fatty acids of broken and intact chloroplasts, 10,000xg, 40,000xg and 144,000xg fractions after $^{14}CO_2$ feeding.

The radioactivity in each fatty acid was determined by fraction collection after gas-liquid chromatography of 20 µl aliquots from a total fatty acid methyl ester solution of 150 µl (6). The total quantity of each lipid and the sum of the fatty acid radioactivities are indicated in Table I.

Lipid	Fraction	Radioactivity in fatty acids					
		16:0	16:1	18:0	18:1	18:2	18:3
		cpm[1]					
MGDG	Broken chloroplast	66	...	22	79	13	13
	Intact chloroplast	35	...	17	31	14	13
	10,000xg	107	...	110	418	35	31
	40,000xg	146	...	56	237	18	11
	144,000xg	26	...	17	43	18	31
DGDG	Broken chloroplast	17	...	0	10	8	7
	Intact chloroplast	13	...	1	8	16	12
	10,000xg	47	...	29	87	19	21
	40,000xg	30	...	18	42	25	17
	144,000xg	10	...	18	26	11	20
PC	Broken chloroplast	36	...	99	386	36	14
	Intact chloroplast	5	...	10	60	10	10
	10,000xg	886	...	1186	7307	231	61
	40,000xg	346	...	537	2679	107	39
	144,000xg	67	...	91	500	29	26
PG	Broken chloroplast	580	252	46	316	35	8
	Intact chloroplast	103	20	33	66	23	16
	10,000xg	769	111	92	406	43	13
	40,000xg	465	72	67	245	29	16
	144,000xg	231	39	32	130	29	30

1 after subtraction of background counts

it represented only a small fraction of the total radioactivity incorporated into PC.

Table II shows the amount of radioactivity incorporated into individual fatty acids of lipids of each fraction. In the MGDG, significant radioactivity was found only in the fatty acids of the 10,000*g* and 40,000*g* fractions. This was concentrated in oleic acid (18:1)[3] with some activity in both palmitic (16:0) and stearic (18:0) acids. The radioactivity incorporated into DGDG fatty acids was significantly lower and once again found mainly in 18:1.

The PC from the 10,000*g* and 40,000*g* fractions contained the highest levels of radioactivity concentrated mainly in 18:1. Radioactivity was found in 16:0 and 18:0 and there was some indication of desaturation of 18:1 with the appearance of significant radioactivity in linoleic acid (18:2) of these two fractions.

In PG the labeling pattern differed in that 16:0 was the most highly labeled fatty acid in all fractions. Significant levels of radioactivity were also found in 18:1 and 16:1. The level of activity in 18:1 in the broken chloroplast fraction, in fact, exceeded the level found in MGDG of the same fraction.

It seems from these results that the major fatty acids incorporated into glycerolipids were 18:1 and 16:0. Specific radioactivities of these two fatty acids were determined in order to estimate their possible turnover rates (Table III). From the specific radioactivities of 16:0 the highest turnover rates may be found in PG in both the chloroplast and high speed fractions. The specific radioactivities are more than double those of the MGDG and PC fractions. In the case of 18:1 the PC and PG fractions have the highest specific radioactivities, both lipids having significantly higher specific radioactivities than MGDG.

DISCUSSION

In previous reports (3, 8) it has been suggested that PC may act as an intermediate in the synthesis and desaturation of fatty acids

[3] Denotes number of carbon atoms:number of double bonds.

Table III. Specific radioactivity of palmitic (16:0) and oleic (18:1) acids from the major leaf glycerolipids of *Vicia faba* following $^{14}CO_2$ feeding.

Fraction	16:0				18:1			
	MGDG	DGDG	PC	PG	MGDG	DGDG	PC	PG
	dpm/μmole fatty acid ($\times 10^{-3}$)							
Broken chloroplasts	9	...[1]	5	19	33	...	134	127
Intact chloroplasts	...	...	...	21	...	...	53	67
10,000xg	39	...	38	88	311	45	652	382
40,000xg	29	...	28	62	174	...	462	489
144,000xg	...	...	8	42	...	...	153	80

[1] indicates specific radioactivity less than 1.0×10^3 dpm/μmole fatty acid

for the galactolipids in higher plants. As chloroplast thylakoids contain very little PC this would imply that the synthesis and desaturation would take place outside the chloroplast or in the envelopes and that it may be necessary for a diglyceride to be transferred to the chloroplast envelopes from the nonchloroplast cytoplasm. Our data (7) supported the findings of Douce (1) that the chloroplast envelope may represent a site of galactosylation of MGDG and DGDG. The high speed fractions separated in these experiments are believed to contain at least half of the envelopes stripped from the chloroplasts during extraction. These fractions contain the major part of the radioactivity found in the MGDG and DGDG fatty acids shortly after $^{14}CO_2$ feeding as well (Tables I and II), and suggest that fatty acids are incorporated into MGDG and DGDG in the envelopes.

The chloroplast fractions (broken and intact) contain very little activity in MGDG, DGDG, and PC indicating that the chloroplast thylakoids are not a major site of incorporation of fatty acids. This is not the case with PG where a large proportion of the activity is found in the broken chloroplast (thylakoid) fraction.

Our results indicate that PG is a major site of incorporation of fatty acid in chloroplasts, mainly 16 carbon fatty acid with some 18:1. Together with the high specific activity of 16:0 this suggests that PG is of major importance in 16:0 synthesis in both the chloroplast and the remaining cytoplasm. The major site of 18:1 incorporation into glycerolipids is outside the chloroplast thylakoid system in conjunction with PC (and to some extent PG). The results presented in this paper, as well as our previous findings (7, 8), are consistent with the hypothesis that MGDG and DGDG are secondary recipients of fatty acids (probably as diglycerides) from phospholipids inside and outside the chloroplast, before and after desaturation.

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