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A Mutant of *Arabidopsis* Deficient in the Chloroplast 16:1/18:1 Desaturase[1]

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ABSTRACT

Leaf tissue of a mutant of *Arabidopsis thaliana* contains reduced levels of both 18-carbon and 16-carbon polyunsaturated fatty acids and increased levels of the 18:1 and *cis*-16:1 precursors due to a single nuclear mutation at a locus designated *fadC*. Analysis of the fatty acid compositions of individual lipids and the kinetics of lipid labeling with [^{14}C]acetate *in vivo* indicate that the mutant lacks activity of the chloroplast glycerolipid ω-6 desaturase. As a result, lipids synthesized by the prokaryotic pathway are not desaturated further than 18:1 and 16:1. Lipids derived from the eukaryotic pathway are desaturated—presumably by the endoplasmic reticulum 18:1 phosphatidylcholine desaturase. However, an increase in the level of 18:1 on all the phospholipids derived from the eukaryotic pathway in leaves of the mutant suggests that the mutation does exert an effect on the composition of extrachloroplast membranes. Synthesis of monogalactosyldiacylglycerol (MGD) by the prokaryotic pathway is reduced 30 to 35% in the mutant and there is a corresponding increase in MGD synthesis by the eukaryotic pathway. This shift in metabolism which results in a more unsaturated MGD pool, may reflect the existence of a regulatory mechanism which apportions lipid synthesis between the two pathways in response to alterations in the physical properties of the chloroplast membranes.

It is now generally accepted that there are two distinct pathways in plant cells for the biosynthesis of glycerolipids and the associated production of polyunsaturated fatty acids (10, 23, 29). Both pathways are initiated by the synthesis of 16:0-ACP[2] in the plastid. 16:0-ACP may be elongated to 18:0-ACP and then desaturated to 18:1-ACP by a soluble desaturase so that 16:0-ACP and 18:1-ACP are the primary products of plastid fatty acid synthesis. In 16:3 species such as *Arabidopsis thaliana*, these thioesters may be used within the chloroplast for the acylation of glycerol-3-P and the subsequent synthesis, via the prokaryotic pathway, of chloroplast lipids including PG, MGD, DGD, and SL (2, 8, 10, 12, 21, 22, 27). Alternatively, they may be converted at the chloroplast envelope to CoA esters (23) which are exported and used mainly in the endoplasmic reticulum for the synthesis of PA by the eukaryotic pathway. Reactions of the eukaryotic pathway give rise to the phospholipids—such as PC, PE, PI, and PG—which are characteristic of the various extrachloroplast membranes (18). In addition however, a proportion of the PC synthesized in the endoplasmic reticulum is returned to the chloroplast envelope where it contributes to the synthesis of thylakoid lipids (6, 23, 29).

In the last few years it has become clear that 16:0 and 18:1 fatty acids are only desaturated to their final products (predominantly 16:3 and 18:3) after incorporation into glycerolipids. The desaturases are assumed to be membrane-bound enzymes, but to date none has been solubilized. For this reason there is little information about the details of ω-6 and ω-3 desaturation reactions in plants. Isolated spinach chloroplasts supplied with [^{14}C]acetate were observed to synthesize 18:1/16:0-MGD which was then sequentially desaturated to 18:3/16:3-MGD (22). In the same way, 18:1 incorporated into PG or SL was desaturated to 18:2 and 18:3 (12, 23, 27). These observations imply the existence of a family of fatty acid desaturases which are located in the chloroplast and which use as substrates glycerolipids produced by the prokaryotic pathway. There is evidence that these desaturases can also act on 18:1- and 18:2-containing chloroplast lipids derived from the eukaryotic pathway (5, 29). However, in the eukaryotic pathway, the predominant conversion of 18:1 to 18:2 and at least some conversion of 18:2 to 18:3 takes place with PC of the endoplasmic reticulum as substrate (20, 23, 25). With the exception of the 18:0-ACP desaturase (17), it has not been possible to obtain information about the chloroplast desaturases by traditional biochemical approaches. Indeed, the ability of intact chloroplasts to synthesize polyunsaturated fatty acids is completely lost when the chloroplasts are broken and is also inhibited in intact chloroplasts which are swollen by mild hypotonic treatment (1). To enlarge the range of approaches available for the study of the properties of the desaturases and the role of lipid unsaturation in membrane function, we have isolated a series of mutants of *Arabidopsis* with specific alterations in leaf fatty acid composition. We have previously described one mutant which does not synthesize *trans*-hexadecenoic acid (3) and another which is deficient in the conversion of 18:2 to 18:3 and of 16:2 to 16:3 (5, 16). Here we report the biochemical charac-

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[2] Abbreviations: ACP, acyl carrier protein; X:Y fatty acyl group containing X carbons with Y *cis*-double bonds; DGD diacyldigalactosylglycerol (digalactosyldiglyceride); MGD diacylgalactosylglycerol (monogalactosyldiglyceride); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SL, sulfoquinovosyldiacylglycerol (sulfolipid); *fadC*, symbol for a gene controlling fatty acid desaturation.

terization of a mutant which is deficient in the conversion of 18:1 to 18:2 and of 16:1 to 16:2.

MATERIALS AND METHODS

Plant Material

The lines of *Arabidopsis thaliana* (L.) Heynh described here were descended from the Columbia wild type. The mutant line LK3 was isolated following mutagenesis with ethyl methane sulfonate (9) and direct identification of mutants by GLC analysis (3). The line was backcrossed to wild type twice before being used for the experiments described here. Plants were grown under continuous fluorescent illumination (150–200 μEm^{-2} s^{-1}) at 22°C on a mixture of perlite: vermiculite:sphagnum (1:1:1) irrigated with mineral nutrients (9).

Extraction Purification and Fatty Acid Analysis of Lipids

Leaf material was frozen in liquid N_2 and lipids extracted as previously described (6). Individual lipids were purified either by a one-dimensional TLC method on $(NH_4)_2SO_4$-impregnated plates (13) or by two-dimensional TLC (24). Lipids were located by light staining with I_2 vapor. Individual lipids separated by TLC were transmethylated with 1 M HCl in methanol after the addition of 17:0 or 14:0 methyl ester as internal standard. The resulting methyl esters were then quantified by GLC (4).

Other Procedures

The labeling of *Arabidopsis* plants with sodium [^{14}C]acetate and the procedures for determining the distribution of radioactivity in lipids and fatty acids have been described (6). Under the conditions used, incorporation of [^{14}C]acetate did not continue beyond 90 min after application of the label. The isolation of protoplasts and the preparation of chloroplast and extrachloroplast membranes were essentially as described (26).

RESULTS

Genetic Analysis

The mutant line LK3 was isolated without selection by screening a mutagenized (M2) population of plants by GLC analysis of fatty acids derived from small leaf samples (3). The line was retained because the analysis revealed a substantial increase in both 18:1 and 16:1, the virtual absence of 16:3 and a significant reduction in the level of 18:3 compared with the wild type (Table I). The mutant is slightly lighter in color but is otherwise indistinguishable from the wild type in appearance and is healthy and vigorous. Unlike the *fadD* mutants we have previously described (5), the fatty acid composition of the LK3 line did not change significantly following growth at a range of temperatures from 4 to 25°C (results not presented).

The F1 progeny of a cross between LK3 and the wild type had approximately twofold higher levels of 16:1 and 18:1 than the wild type (Table I). Thus, the wild-type allele should probably be considered as incompletely dominant. The F2 progeny derived from self-fertilization of several F1 plants were grown and the fatty acid composition determined from leaf samples of 221 plants. Of these, 170 had essentially the same fatty acid composition as the wild type while 51 were indistinguishable from the original LK3 line in the composition of both 16-carbon and 18-carbon fatty acids. This segregation pattern is a reasonable fit ($X^2 = 0.44$; $P > 0.4$) to the 3:1 hypothesis. The mendelian inheritance pattern and the cosegregation of elevated 16:1 with elevated 18:1 in the F2 population indicate that the altered fatty acid composition of the mutant is caused by a single nuclear mutation at a locus we have designated *fadC*.

Table I. *Fatty Acid Composition of Total Leaf Lipids of Mutant and Wild-Type Arabidopsis Grown at 22 °C*

Fatty Acid	WT[a]	F1 (WT × LK3)	LK3
		mol % ± s.d. (n=10)	
16:0	13.0 ± 0.8	12.3 ± 0.5	13.9 ± 0.3
16:1-*cis*	1.5 ± 0.2	3.7 ± 0.3	11.2 ± 0.7
16:1-*trans*	3.6 ± 0.4	2.5 ± 0.3	3.8 ± 0.5
16:2	1.7 ± 0.5	0.3 ± 0.1	0.5 ± 0.2
16:3	15.7 ± 1.0	14.0 ± 1.3	0.2 ± 0.1
18:0	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.2
18:1	2.4 ± 0.4	5.8 ± 0.8	16.1 ± 1.0
18:2	12.3 ± 0.6	16.7 ± 0.5	16.5 ± 0.2
18:3	49.1 ± 1.4	43.9 ± 1.7	37.0 ± 1.5

[a] Wild type.

Biochemical Characterization

In the mutant, decreased levels of 18:3 and of 16:3 fatty acids are accompanied by increased 18:1 and 16:1, respectively (Table I). Although alternative hypotheses may be considered (see below), the simplest explanation of these observations is that the mutant is deficient in the activity of a fatty acid desaturase which, in the wild type, introduces a double bond at the ω-6 position of 16- and 18-carbon acyl groups. Because MGD derived from the prokaryotic pathway is the presumed substrate for 16:3 synthesis (22, 23), we conclude that this desaturase is located in the chloroplast. Almost no 16:3 is detectable in the total leaf lipids (Table I), indicating that the desaturase is essentially nonfunctional—at least on 16:1 acyl chains. Thus, it is likely that the 18:2 and 18:3 present in the mutant are derived largely via the endoplasmic reticulum 18:1-PC desaturase of the eukaryotic pathway (23, 25).

The fact that 18:1 and 16:1 accumulate to high levels in the mutant rather than the Δ9,15-isomer of 18:2 and the Δ7,13 isomer of 16:2, indicates that the ω-3 desaturase which normally desaturates both Δ9,12-18:2 and Δ7,10-16:2 (5) cannot use 18:1 or 16:1 as an effective substrate, *i.e.* desaturation at the ω-3 position requires the presence of a double bond at the ω-6 position (but see below).

Fatty Acid Composition of Individual Lipids

Considerably more information about the desaturation step controlled by the *fadC* gene product can be deduced from an

analysis of the fatty acid composition of individual lipids extracted from leaf tissue of wild type and mutant plants (Table II). The data show that there is no major change in the proportions of the various polar lipids in the mutant. The largest changes were a 9% decrease in the amount of MGD and a corresponding increase in the amount of DGD. However, all of the chloroplast polar lipids show a substantial decrease in the level of 18:3 fatty acids and an increase in the level of 18:1. In addition, 16:3 is essentially absent from MGD of the mutant, while *cis*-16:1 becomes the major unsaturated 16-carbon fatty acid in this lipid. However, in the wild-type, 16:1+16:2+16:3 in MGD account for 15.1% of all the fatty acids in the polar lipids, but they represent only 8.6% in the mutant (Table II). Furthermore, small amounts of *cis*-16:1 were found in PC and PE of the mutant even though 16:3 is not a component of these lipids in wild-type *Arabidopsis*. These data suggest the possibility that some 16:1-MGD is broken down in the mutant and fatty acids reincorporated into phospholipids. However, the *cis*-16:1 in PC and PE constitute only 1.2% of the total polar lipid fatty acids, indicating that such recycling is not the principal reason for the reduced level of 16-carbon fatty acids on MGD (see below).

In *Arabidopsis* and other 16:3 plants, MGD contains 16:3 exclusively at position *sn*-2 of the glycerol. On the other hand, chloroplast PG, which constitutes about 85% of the total leaf PG (6), contains 18:3 only at position *sn*-1 of the glycerol. Therefore the observation that 16:3 of MGD and 18:3 of PG are both strongly reduced in the mutant indicates that the *fadC* gene product controls desaturation of fatty acids at both positions of the glycerol backbone. However, when 16:0 is present at *sn*-2 of PG, *sn*-1 of DGD, or at either position of SL, it is not desaturated. Thus, the ω-6 desaturase apparently accepts as substrates only fatty acyl groups with a double bond at ω-9. If, as we suggest, the *fadC* gene product is the chloroplast ω-6 desaturase *per se*, then these results indicate that it acts on any ω-9 acyl chain in the chloroplast with little or no specificity for the length of the chain (16- or 18-carbon), its point of attachment to the glycerol backbone (*sn*-1 or *sn*-2), or for the lipid head group.

Desaturation of 18:1 by the desaturase associated with the eukaryotic pathway takes place predominantly on PC of the endoplasmic reticulum (23, 25). For this reason, only those molecules of MGD, DGD, SL, and PG made by the prokaryotic pathway would be expected to be affected by the mutation at the *fadC* locus. This is largely true inasmuch as the composition of the lipids which are synthesized predominantly by the prokaryotic pathway in the wild type (PG and MGD, ref. 6) are most drastically altered in the mutant (Table II). However, in addition, the phospholipids PC, PE, and PI, which are derived entirely from the eukaryotic pathway, show a twofold increase in the amount of 18:1, compared with the wild type. Taken together with the analogous results for a mutant deficient in the chloroplast ω-3 desaturase (3), they suggest that both chloroplast and extrachloroplast desaturases may determine the level of unsaturation of phospholipids in the extrachloroplast membranes.

It is interesting to note that the eukaryotic phospholipids (PC, PE, and PI) from the mutant all show an increase in the level of 18:2 relative to the wild type (Table II). Although this effect is small, it appeared consistently in at least four independent batches of plants. However, it is not clear how this effect is related to the loss of ω-6 desaturase activity in the mutant.

Because chloroplast PG is derived exclusively from the prokaryotic pathway, while PG in other cell membranes is formed by the eukaryotic pathway, it seems probable that 18:2 and 18:3 in the total leaf PG (Table II) represent extrachloroplast PG. In order to determine an upper limit on the activity of the ω-6 desaturase on 18:1 in chloroplast membranes of the mutants, we analyzed the fatty acid composition of PG from chloroplast and extrachloroplast membranes. Analysis of PG from the chloroplast membranes of LK3 showed considerably lower levels of 18:2 and 18:3 fatty acids (Table III) than were found in the total leaf PG, while PG from the extrachloroplast membranes of LK3 contained considerable 18:2 and 18:3. This crude extrachloroplast mem-

Table II. *Fatty Acid Composition of Leaf Lipids from Wild-Type and Mutant Arabidopsis Grown at 22 °C*

Fatty Acid	MGD		DGD		SL		PG		PC		PE		PI	
	WT	LK3	WT	LK3	WT	LK3	WT	LK3	WT	LK3	WT	LK3	WT	LK3
							mol %							
16:0	1.1	3.1	11.7	12.0	40.3	36.7	30.8	28.7	22.1	21.8	29.6	31.0	43.5	42.6
c16:1	0.7	21.4	—[a]	4.3	—	—	—	—	0.7	4.7	—	2.6	—	—
t16:1	—	—	—	—	—	—	16.1	19.8	—	—	—	—	—	—
16:2	2.0	1.2	0.7	1.0	—	—	—	—	—	—	—	—	—	—
16:3	33.7	0.1	3.2	0.1	—	—	—	—	—	—	—	—	—	—
18:0	—	0.3	0.9	1.2	1.6	2.8	1.6	2.0	2.2	2.0	2.2	2.2	2.9	3.6
18:1	0.7	29.7	1.6	16.3	2.2	21.3	4.1	36.1	6.1	12.4	2.9	7.8	3.7	7.6
18:2	2.7	1.9	4.4	1.8	11.6	9.7	8.9	5.7	35.3	35.9	34.4	38.2	24.0	26.5
18:3	59.1	42.0	77.5	63.2	44.3	29.5	38.5	7.7	33.6	22.9	30.9	18.0	25.9	19.7
Proportion of leaf polar lipids (%)														
	41.5	37.8	12.8	15.7	3.0	3.6	9.7	9.4	19.3	18.4	10.0	11.6	3.7	3.5

[a] Dashes indicate that the acyl group was not detected.

Table III. *Fatty Acid Composition of Phosphatidylglycerol of Chloroplast and Extrachloroplast Membranes from Wild-Type and Mutant Arabidopsis*

Fatty Acid	Chloroplasts				Extrachloroplast Membranes			
	Measured		Corrected[a]		Measured		Corrected[a]	
	WT[b]	LK3	WT	LK3	WT	LK3	WT	LK3
	mol %							
16:0	18.9	18.9	19.4	19.7	24.0	25.2	33.0	34.1
16:1-*cis*	0	1.3	0	0	2.9	7.1	4.6	4.5
16:1-*trans*	35.1	31.9	37.2	34.5	8.3	8.0	0	0
16:3	0.9	0	0	0	5.8	0	0	0
18:0	2.1	0.9	2.2	0.9	6.4	4.7	10.0	7.6
18:1	6.0	41.8	6.3	43.2	8.7	18.1	12.1	4.5
18:2	7.9	2.1	8.0	1.7	19.0	23.0	28.3	37.1
18:3	27.8	2.5	26.9	0	23.0	12.7	11.9	12.1

[a] Corrections for cross-contamination between samples and acyl migration from other chloroplast lipids are described in the text. [b] Wild type.

brane preparation was contaminated to a small extent with chloroplast membranes. In addition, we have shown elsewhere (7) that the procedures used in preparing mesophyll protoplasts result in some migration of fatty acids between different lipids. An approximate correction for this artefact can be made by assuming that the trace of 16:3 (in wild type PG) or *cis*-16:1 (in LK3 PG) reflects the extent of contamination by fatty acids from MGD. Similarly, the level of *trans*-16:1 in the PG of extrachloroplast membranes can be taken as a measure of contamination by chloroplast PG. When these corrections are made (Table III), it is clear that the chloroplast PG of LK3 contains levels of 18:2 + 18:3 which are less than 1% of the levels in chloroplast PG of the wild type; whereas the amounts of 18:2 and 18:3 in PG of the extrachloroplast membranes are not reduced in the mutant.

During our analysis of the fatty acid compositions of leaf lipids, we observed that the peak representing 18:2 in MGD and DGD of the mutant, but not the wild type, could be resolved into two peaks of approximately equal size. On a 15 m × 0.53 mm Supelcowax 10 column, the retention time of the first peak was the same as that of 18:2 from the wild type, while the second peak eluted 0.2 min later (Fig. 1). The 16:2 from MGD of the mutant also eluted 0.2 min later than the Δ7,10 isomer of the wild type and the GC trace from a mixture containing methyl esters of LK3 and wild-type MGD showed two clearly separated peaks of 16:2 (data not shown). When fatty acid methyl esters derived from MGD of LK3 were separated by argentation-TLC, an additional compound not present in MGD of the wild type was detected slightly above 18:3 and well below the band containing Δ9,12-18:2. This compound was eluted from the silica gel and shown to have the same retention time during GC as the peak eluting after the normal 18:2. Analysis by GC-MS demonstrated that the compound was an isomer of 18:2. Although we did not determine the positions of the double bonds in this 18:2 isomer, we consider that it is most probably Δ9,15-18:2 formed in small amounts by the action of an ω-3 desaturase on 18:1. In the same way the mutant would contain Δ7, 13-16:2. On the basis of this assumption, it is possible to estimate the specificity of the ω-3 desaturase for 18:2 over 18:1 (and for 16:2 over 16:1) as its substrate. In the wild type, fatty acid analyses (Table II) and the data from ^{14}C-labeling (Fig. 3) indicate that more than 90% of the 18:2 and 16:2 synthesized on MGD and DGD is desaturated by the ω-3 desaturase(s) to 18:3 or 16:3. In contrast, only 2 to 5% of the 18:1 present on these lipids from LK3 plants was converted to Δ9,15-18:2 (data not shown), indicating a 20- to 40-fold preference of the enzyme for 18:2 over 18:1 and 16:2 over 16:1.

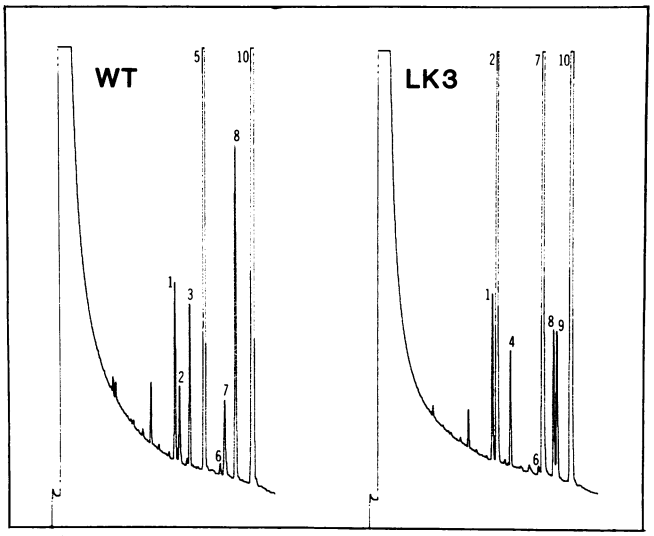

Figure 1. Gas chromatographic separation of fatty acid methyl esters derived from MGD of wild type and mutant *Arabidopsis*. Peaks identified in the wild type are 16:0 (1); Δ7-16:1 (2); Δ7,10-16:2 (3); Δ7,10,13-16:3 (5); 18:0 (6); Δ9-18:1 (7); Δ9,12-18:2 (8); Δ9,12,15-18:3 (10). Additional peaks in the mutant LK3 are tentatively identified as Δ7,13-16:2 (4) and Δ9,15-18:2 (9) (see text). The retention time for 18:3 was 13.5 min.

Labeling of Leaves

In wild-type *Arabidopsis*, 70 to 75% of the MGD is derived from the prokaryotic pathway and contains an unsaturated 16-carbon fatty acid at the *sn*-2 position of the glycerol and an unsaturated 18-carbon fatty acid at the *sn*-1 position (6). MGD synthesized by the eukaryotic pathway contains almost exclusively 18-carbon fatty acids, although a small amount of 16:0 is present at the *sn*-1 position (6). Consistent with these observations, MGD from wild-type plants contained 36.4% 16:1 + 16:2 + 16:3 (Table II). However, these fatty acids account for only 23% of the MGD fatty acids in the mutant, indicating that less than half of the chloroplast MGD is produced by the prokaryotic pathway. This situation may reflect increased turnover in the mutant of the prokaryotic MGD which contains high levels of monoenoic fatty acids. Alternatively, a decrease in the flux entering the prokaryotic pathway might be involved. To help distinguish these possibilities and to extend the comparison between lipid biosynthesis in the mutant and that in the wild type, we labeled wild type and mutant plants with [^{14}C]acetate and then followed redistribution of radioactivity in lipid acyl groups during the subsequent 144 h. The kinetics of labeling in the wild type

are similar to those found previously (6) and are consistent with the parallel operation of the prokaryotic and eukaryotic pathways of lipid synthesis. We have demonstrated elsewhere (6) that the label found in MGD at the beginning of the experiment represents MGD derived from the prokaryotic pathway, while the increase in MGD label at longer times reflects synthesis of this lipid via the eukaryotic pathway. On this basis, the data for LK3 in Figure 2 do indeed demonstrate a substantial decline, compared with the wild type, in MGD synthesis by the prokaryotic pathway and a corresponding increase in the synthesis of this lipid by the eukaryotic pathway. Previous analysis of another lipid mutant of *Arabidopsis* (14) has demonstrated that lipid synthesis in leaves is tightly regulated by demand for particular lipids for membrane biogenesis. Similarly, we believe that in LK3, the altered balance between the two pathways for MGD synthesis reflects the operation of a regulatory mechanism which is responsive to the physical properties of chloroplast membranes.

Analysis of the distribution of ^{14}C among the fatty acids from each lipid (Fig. 3) confirmed that it was the chloroplast lipids produced by the prokaryotic pathway that were mainly affected by the mutation at *fadC*. In MGD and DGD, for example, early labeling involved only 16:0, 16:1, and 18:1 fatty acids in the mutant. Labeled 18:3 only became a significant component of these lipids 5 to 10 h after application of [^{14}C]acetate when fatty acids derived from the eukaryotic pathway begin to be used for MGD and DGD synthesis (Fig. 2). Similarly, ^{14}C-18:1 incorporated into PG shows essentially no conversion to more highly unsaturated species. In contrast, the labeling kinetics of the other phospholipids are similar to the labeling kinetics of the corresponding lipids from wild type leaves.

Fatty Acid Composition of Roots and Seeds

In plant roots, which contain a predominance of extraplastid membranes, and seeds, which contain large amounts of triglycerides, the prokaryotic pathway does not contribute significantly to lipid synthesis. Comparison of the overall fatty acid composition of the roots and mature seeds from the mutant and wild type showed no detectable difference in the amount of 18:1 in the lipids of either of these organs (Table IV). These observations are consistent with other results, presented above, indicating that the *fadC* locus controls activity of a prokaryotic pathway desaturase. The lack of an effect on eukaryotic lipids suggests that changes seen on eukaryotic lipid desaturation in leaves is not due to an effect of the *fadC* gene product on the desaturases associated with the eukaryotic pathway.

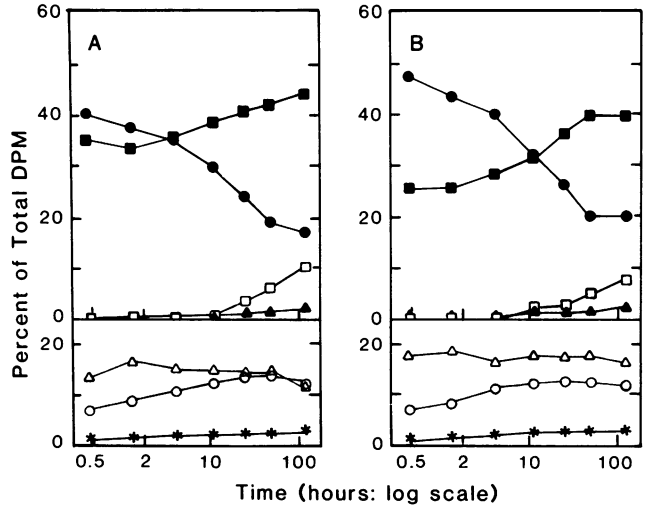

Figure 2. Redistribution of radioactivity among the polar leaf lipids of wild type (A) and mutant (B) *Arabidopsis*. Plants were incubated in the light and labeled with sodium [^{14}C]acetate at time zero. Lipids from samples of leaves were separated by TLC. (■), MGD; (●), PC; (□), DGD; (▲), SL: (△), PG; (○), PE; (*), PI.

DISCUSSION

With the exception of the stearyl-ACP desaturase (17), the desaturation reactions in higher plants require glycerolipid substrates and appear to involve membrane bound enzymes (1, 5, 11, 22, 25, 27). A detailed mechanism is not known for any plant desaturase but by analogy to the mammalian enzymes it is assumed that to be active the desaturases must be physically associated with electron transport components and associated reductases (19, 28). Solubilization, purification, and reconstitution of activity has not been achieved for any of the plant glycerolipid desaturases, and the enzymes involved in desaturation of chloroplast lipids have been found to be especially labile (1). For this reason, it has not been possible to characterize the *fadC* mutant by direct enzyme assay. However, the fatty acid analysis of leaf lipids (Table II) and the results of [^{14}C]acetate labeling experiments (Figs. 2 and 3) support the conclusion that the mutant phenotype is the result of a specific deficiency in activity of a chloroplast ω-6 desaturase which acts on 18:1 and *cis*-16:1 acyl chains of lipids derived from the prokaryotic pathway.

Analysis of the effects of the *fadC* mutation and the previously described *fadD* mutation (5) on lipid composition have provided insights into the substrate specificity of the desaturases controlled by these loci. Both the ω-3 and ω-6 desaturases act on 16-carbon as well as 18-carbon fatty acids and therefore appear to determine the site of desaturation relative to the methyl end of the acyl chain. Both enzymes will act on acyl groups at either the *sn*-1 and *sn*-2 positions of the glycerol backbone. Finally, both enzymes appear to effect desaturation of all the major glycerolipids of the chloroplast membranes. This conclusion is based on the assumption that there is no significant transfer of acyl groups between chloroplast lipids. In support of this, it has been demonstrated that isolated spinach chloroplasts supplied with [^{14}C]acetate have been induced by the addition of appropriate lipid precursors, to accumulate label in the acyl groups of either MGD (22), PG (21, 27), or SL (12). In each of these studies, desaturation of the labeled fatty acids occurred without the involvement of any other glycerolipid. From these observations, we infer that the ω-6 and ω-3 desaturations involve enzymes that act on all the chloroplast glycerolipids rather than desaturation on a single substrate with subsequent transfer of fatty acids to the other lipids.

A major difference between the ω-6 and the ω-3 desaturases is the extent to which they contribute to the desaturation of lipids of the extrachloroplast membranes. In the *fadD* mutant,

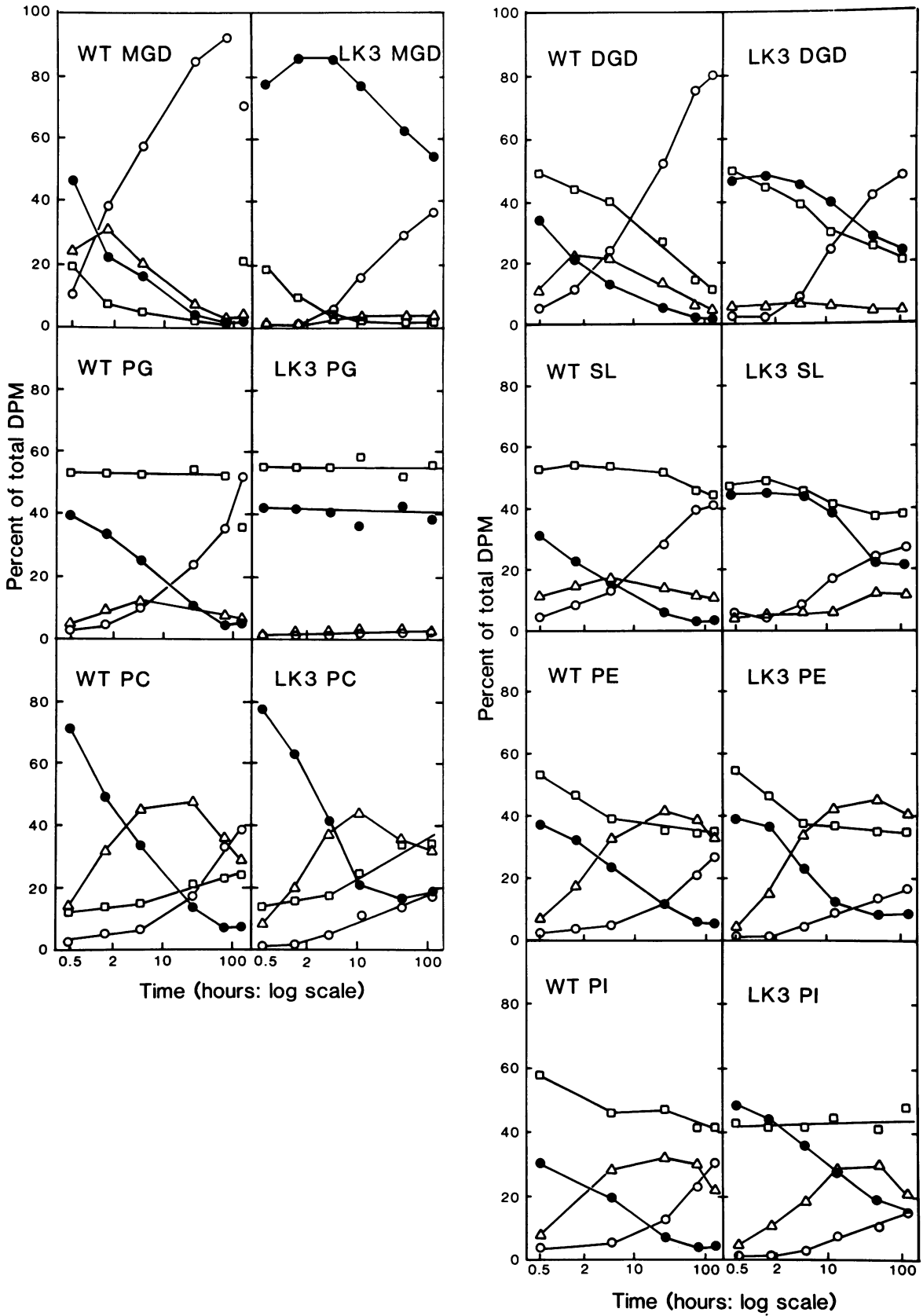

Figure 3. Redistribution of radioactivity among fatty acids of individual lipids from wild type and mutant *Arabidopsis*. Radioactive lipids were obtained as described in Figure 2. Fatty acid methyl esters were prepared and separated by argentation TLC. (□), saturated; (●), monoenoic; (△), dienoic; (○), trienoic.

Table IV. *Fatty Acid Composition of Roots and Seeds of Wild-Type and Mutant Arabidopsis*

Fatty Acid	Seeds		Roots	
	Wild type	LK3	Wild type	LK3
	weight % ± SD[a]			
16:0	9.2 ± 0.8	9.8 ± 1.0	26.3 ± 3.2	25.8 ± 2.5
16:1			0.6 ± 0.2	0.9 ± 0.3
18:0	3.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.8	2.8 ± 0.6
18:1	12.4 ± 0.8	11.2 ± 0.6	5.6 ± 2.6	6.3 ± 3.1
18:2	30.4 ± 1.1	30.0 ± 1.1	37.7 ± 3.4	34.3 ± 5.0
18:3	20.1 ± 1.4	20.4 ± 1.8	22.0 ± 2.4	21.9 ± 4.7
20:1	21.7 ± 0.8	22.3 ± 1.2		
22:1	2.0 ± 0.1	2.0 ± 0.2		

[a] $n = 10$.

deficient in activity of an ω-3 desaturase, the ratio of 18:3 in the mutant to that in the wild type was similar for all the major leaf polar lipids (5). This observation is consistent with the existence of at least two 18:2 desaturases, one of which—the *fadD* gene product—contributes equally to the desaturation of chloroplast and other membrane lipids. In contrast, the results reported here indicate that the *fadC* gene product is entirely responsible for desaturation of 18:1 on lipids of the prokaryotic pathway while desaturation of eukaryotic pathway lipids is carried out predominantly by one or more other enzymes such as the 18:1-PC desaturase of the endoplasmic reticulum (25). Nevertheless, the twofold increase of 18:1 in extrachloroplast lipids (Table II)—particularly in PE which is not a constituent of chloroplast membranes (15)—reveals a role of the *fadC* locus in determining the extent of unsaturation of membranes outside the chloroplast. One possibility that is consistent with the results presented here and in a previous study of the *fadD* mutant (5) is that there is significant transfer of acyl groups from the chloroplast to the extrachloroplast membranes.

The accumulation of 18:1 and 16:1 in the *fadC* mutant (rather than ω-9, ω-3 dienoic fatty acids) indicates that monoenoic fatty acids are not suitable substrates for the ω-3 desaturase. The simplest explanation is that while the ω-3 desaturase determines the position for insertion of the double bond relative to the methyl end of the molecule (5), it has an additional requirement for existing double bond at ω-6. Similarly, the fact that the ω-6 desaturase does not act on 16:0 at *sn*-2 of SL and PG indicates that this enzyme requires there to be an existing double bond at ω-9. These suggestions are consistent with the findings of Howling *et al.* (11) for the desaturase of *Chlorella* presented with various monoenoic fatty acid substrates. Thus, our results are consistent with a model for the ω-6 and ω-3 chloroplast desaturases as enzymes which reside mainly in the hydrophobic region of the thylakoid (or envelope) bilayer so that they have little or no interaction with the hydropilic headgroups of the membrane lipids and which desaturate any fatty acids which meet the requirement of having a specific distance between an existing double bond and the methyl end of the acyl chain.

The mutation at the *fadC* locus has a very large effect on the degree of unsaturation of the photosynthetic membranes of the LK3 line. The average number of double bonds per glycerolipid molecule was 4·57 in chloroplasts from the wild type but only 3·13 in chloroplasts from LK3 (data not shown). In MGD, the major thylakoid lipid, the number of double bonds per molecule fell from 5·78 in the wild type to 3·69 in the mutant (Table II). This change in the degree of thylakoid membrane unsaturation has no pronounced effects on the growth rate or vigor of the mutant under our standard growth conditions. There is, however, one indication from the biochemical studies reported here that lipid biosynthesis in the mutant may be regulated to ameliorate the effect of the *fadC* mutation. Compared with the wild type, the LK3 line shows a 30 to 35% decline in synthesis of MGD via the prokaryotic pathway and a corresponding increase in MGD synthesis by the eukaryotic pathway (Table II: Fig. 2). The net effect is that a greater proportion of the acyl groups on MGD is desaturated by the endoplasmic reticulum 18:1 desaturase (25) so that more MGD containing 18:3 is produced (Fig. 3). It can be calculated from the data in Table II that in the absence of such a shift in metabolism, MGD from leaves of the mutant would contain an average of less than 2·8 double bonds per molecule.

Since we do not yet understand what factors mediate the partitioning of lipid synthesis between the two pathways, we are not able to definitively confirm that this altered balance between the two pathways of MGD synthesis is a regulated response to the loss of chloroplast ω-6 desaturase activity. However, these observations are consistent with a previous analysis of *Arabidopsis* mutants deficient in acyl-ACP:*sn*-glycerol-3-phosphate acyltransferase (a key enzyme of the prokaryotic pathway) in which it was apparent that demand for a balanced complement of different lipids necessary for the correct assembly of chloroplast membranes can indeed strongly regulate lipid metabolism in leaf cells (14).

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