Plant Physiol. (1988) 87, 904–910
0032-0889/88/87/0904/07/$01.00/0

The Effect of Temperature on the Level and Biosynthesis of Unsaturated Fatty Acids in Diacylglycerols of *Brassica napus* Leaves[1]

Received for publication February 9, 1988 and in revised form April 4, 1988

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ABSTRACT

Experiments on the effects of temperature on the levels of unsaturated fatty acids and their rates of desaturation in *Brassica napus* leaf lipids have shown that significant differences occur in the composition of all diacylglycerols in the leaf between plants grown at high and low temperatures. In the major thylakoid diacylglycerols, monogalactosyl-diacylglycerol and digalactosyldiacylglycerol, not only is there an increase in the level of unsaturation at low temperatures, but there is a change in the balance between molecular species of chloroplastic origin (16/18C) and cytosolic origin (18/18C). Radioactivity tracer data indicate that at low temperatures there are two distinct phases of desaturation in the fatty acids of the major diacylglycerols of these leaves. A rapid phase, which appears in plants grown at low temperatures and results in the desaturation of palmitic acid to hexadecadienoic acid and oleic acid to linoleic acid may explain the high levels of unsaturated fatty acids found in the leaf diacylglycerols from plants grown at low temperatures. The appearance of this rapid phase is controlled by the temperature at which the plant is grown and is not subject to rapid variations in environmental temperature.

A common phenomenon found in many prokaryotes, plants, and animals is the ability of an organism to adapt to low environmental temperatures by increasing the level of unsaturation in the fatty acids of membrane diacylglycerols (8, 12, 13). It is generally believed that the increase in unsaturation affords the organism some protection from low temperature damage and/or allows the organism to optimize membrane-bound or dependent metabolic reactions. It is possible that some organisms also adapt to higher temperatures by lowering the level of unsaturation of their fatty acids. These changes in levels of unsaturation are thought to affect the general fluidity of membranes and/or to have selected or localized effects on specific membrane components such as photosynthetic electron transport (9, 10).

In plants, no satisfactory theory has been advanced to explain how this modification in unsaturation is accomplished. Harris and James (2) proposed that the increased solubility of O_2 at low temperatures would increase desaturase activity because O_2 is a substrate in this reaction. Others (5, 6) have suggested that modifications in membrane fluidity induced by changes in environmental temperatures result in conformational changes in membrane-bound enzymes, thus altering their activity. A third possibility (8, 12, 16) is that the lower temperatures result in changes in the transcription, translation, or post-translational modification of the enzyme resulting in either higher levels of production of the enzyme or increased enzyme efficiency or activity. The desaturase enzyme might also be high temperature labile or controlled by a thermally labile modulator as has been proposed in *Bacillus* (1).

Research in this area in relation to the production of the major diacylglycerols in leaves has been hampered by the inability to isolate the desaturase enzymes and perform standard *in vitro* kinetic analyses. *In vivo* analyses have been difficult because until recently (4, 15) the understanding of the metabolic pathways of the major chloroplast diacylglycerols has been confused. It is now clear that in all plants the pathways of fatty acid biosynthesis and incorporation into the galactosylglycerols of the thylakoids are complex, involving both cytosolic and chloroplastic components. In 16:3[2] plants there are two distinct pathways. One is a purely chloroplastic pathway that involves unsaturation of fatty acids in association with galactosyldiacylglycerols (or the immediate diacylglycerol precursor) to form molecular species containing one 16 and one 18 carbon fatty acid. In addition, a second cytosolic pathway results in the formation of highly unsaturated molecular species containing two 18C fatty acids. In 18:3 plants, the longer cytosolic pathway involving PC is the predominant pathway while the chloroplastic pathway is of reduced importance and does not involve the unsaturation of 16:0 to 16:3 in galactosylglycerols.

Our data suggest that it is now possible to determine rates of unsaturation of fatty acids in this chloroplastic pathway in 16:3 plants without interference from the cytosolic pathway. We are therefore able to determine the effects of growth temperature and sudden variations in environmental temperature on rates of fatty acid desaturation more accurately than has previously been thought possible.

The data presented here describe the effects of low temperature on *Brassica napus* fatty acid unsaturation using *in vivo* radioactive ^{14}C tracer. Our results show that the theories put forward to explain the increased levels of unsaturated fatty acids at low temperatures are not sufficient. We present possible alternative explanations for this phenomenon.

[1] This work was supported by Operating and Strategic Grants from the Natural Sciences and Engineering Research Council of Canada.

[2] Abbreviations: 16:3, hexadecatrienoic acid; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; SL, sulphoquinovosyldiacylglycerol; 16:0, palmitic acid; 16:1, palmitoleic acid; 16:2, hexadecadienoic acid; *trans*-16:1; *trans*-Δ^3-hexadecenoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

MATERIALS AND METHODS

Brassica napus (var. Tower) plants were grown in growth chambers set at 5, 10, 20, or 30°C under fluorescent/incandescent light intensities of approximately 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 16 h day/8 h night regimes.

Leaves were harvested and offered 1 mCi of $^{14}CO_2$ for 5 min in plexiglass chambers in the same growth chambers under the same light conditions and temperatures. Incubations were carried out in the same chambers after removal of all remaining free $^{14}CO_2$. Lipids were extracted from leaves, purified, and separated as previously described (7, 17). The molecular species of MGDG and DGDG were separated by argentation TLC and analyzed by GLC (Packard model 7300) using a megabore capillary column DB-225 (30 m × 0.53 mm, film thickness 1.0 μm) (J & W Scientific). The initial column temperature was maintained at 170°C for 10 min and then was increased to 210°C at the rate of 2°C/min. with a final hold for 5 min. The carrier gas (helium) flow was regulated at a constant column pressure in the range of 12 to 14 psi and the make-up gas flow was set at 8 psi. The effluent stream was split, and fatty acid fractions were collected and counted by scintillation spectrophotometry as previously described (14).

RESULTS

Diacylglycerol and Chl Composition of Leaves from B. napus Grown at Different Temperatures. Table I contains data on the diacylglycerol composition of *B. napus* leaves grown at four different temperatures. With lower temperatures, the general trends show a relative decrease in the chloroplast components, MGDG and SL, compared to the cytosolic diacylglycerols, PC and PE. Chloroplastic DGDG increased relative to the other chloroplast diacylglycerols, and there appears to be little change in the level of PG that was found in both the cytosol and chloroplast compartments.

Table II shows the effect of temperature on Chl content of the leaves relative to other chloroplast diacylglycerols. While the Chl *a/b* ratio appears to have increased significantly at 5°C, at other temperatures the Chl *a/b* ratios and total Chl relative to the diacylglycerol content did not vary significantly.

Table I. *Diacylglycerol Composition of B. napus Leaves Grown at Different Temperatures*

Standard deviations are in parentheses, $n = 4$.

Lipid	Growth Temperature (°C)			
	5	10	20	30
	mol %			
MGDG	37.8 (2.9)	40.2 (2.5)	42.8 (3.2)	44.6 (0.8)
DGDG	18.1 (1.9)	17.0 (1.0)	17.2 (1.0)	16.4 (1.8)
PC	18.1 (0.7)	18.2 (0.2)	15.2 (1.8)	14.7 (0.9)
PE	11.3 (0.4)	10.7 (0.8)	9.2 (0.9)	8.1 (0.5)
PG	9.8 (1.0)	9.7 (1.3)	9.7 (0.5)	9.3 (1.0)
SL	4.9 (0.6)	4.2 (0.4)	5.9 (0.4)	6.9 (0.5)

Table II. *Lipid:Chl and Chl a/b Ratios in B. napus Leaves Grown at Different Temperatures*

Standard deviations are in parentheses, $n = 4$.

Growth Temperature	Lipid/Chl Ratio with Lipid				
	MGDG	DGDG	PG	SL	Chl *a/b*
°C	*molar ratio*				
5	3.4 (0.2)	1.7 (0.3)	0.9 (0.1)	0.4 (0.1)	5.0 (1.0)
10	2.7 (0.4)	1.1 (0.2)	0.7 (0.2)	0.3 (0.1)	2.9 (0.4)
20	2.3 (0.4)	0.9 (0.1)	0.5 (0.1)	0.3 (0.1)	3.1 (0.4)
30	2.5 (0.4)	0.9 (0.2)	0.5 (0.1)	0.4 (0.1)	2.8 (0.3)

Fatty Acid Composition of the Major Leaf Diacylglycerols. The fatty acid compositions of the major leaf diacylglycerols from leaves grown at 5, 10, 20, and 30°C are shown in Table III. The trend in all the diacylglycerols was an increase in desaturation at lower temperatures with the exception of *trans*-16:1 in PG which decreased. In MGDG, there was an apparent decrease in the level of 18:3, however, this was compensated for by an increase in the level of 16:3, the total trienoic acid content increasing at lower temperatures.

The molar distribution of 16/18C and 18/18C molecular species of MGDG is shown in Table IV. The changes in levels of 18:3 and 16:3 described above are the result of changes in the relative quantities of 16:3/18:3 and 18:3/18:3 molecular species. At lower temperatures (5°C in comparison to 30°C), the relative contribution of 16:3/18:3 synthesized through the chloroplastic pathway increased by almost 20%, from 61 up to 80%, while the level of 18:3/18:3 decreased from 22% to only 15%. At higher temperatures, significant quantities (up to 16%) of other than hexaene molecular species occurred, while at 5°C 95% of the molecular species were hexaenes.

The total 16/18C and 18/18C molecular species are compared in Table V for each temperature. While the trend in MGDG at lower temperatures was toward an increase in 16/18C chloroplast species, in DGDG the reverse was the case. This suggests a major effect of temperature, not only on the levels of desaturation in the galactosyldiacylglycerols but a change in the biosynthetic pathways favoring the retention of the most highly unsaturated chloroplastic molecular species of MGDG, a decrease in the 16:0/18:3(2) DGDG, and an accumulation of cytosolic 18/18C molecular species in DGDG.

Effect of Growth Temperature on Rate of Desaturation. Table VI contains data from experiments in which plants were grown at four different temperatures and after a brief period of temperature equilibration (30 min) were offered $^{14}CO_2$ at 20°C and incubated at the same temperature for up to 60 min. The results show that plants grown at lower temperatures were able to desaturate fatty acids more rapidly than plants grown at higher temperatures. They further show that at lower growth temperatures there was a rapid, within 5 to 10 min, conversion of 16:0 to 16:2 and 18:1 to 18:2. This rapid phase of desaturation occurred predominantly at 5 and 10°C and appears to have been very much reduced or absent at 30°C. The degree of desaturation to 16:2 and 18:2 after only 5 to 10 min of incubation suggests a process or pathway at low temperatures quite distinct from those found at 30°C. Despite the presence of this rapid desaturation at low growth temperatures, significant levels of radioactivity remained in 16:0 after 30 to 60 min of incubation. This suggests two distinct processes or phases in the desaturation of 16:0 and 18:1 in MGDG in plants grown at low temperatures.

Table VII contains data from experiments designed to determine if this rapid phase is a normal part of leaf development in plants grown at different temperatures or if it is related to the physiological age of the leaves. Plants were grown at 10, 20, and 30°C, and leaves were sampled over a period of 26 d after their appearance at d 1 as folded, unexpanded leaves. The results suggest, with the exception of the very young leaves developing at 20°C, that the appearance of the rapid phase is dependent on the temperature of growth rather than stage of development or physiological age. The one result obtained with 30°C-grown plants suggests that this phase may be quickly lost during development of the leaf at higher temperatures. This leaf showed the rapid phase of desaturation in both 16 and 18C fatty acids in MGDG and 18C fatty acids in PC (see below).

Effect of Incubation Temperature on Rate of Desaturation. In the previous experiments, plants were grown at different temper-

Table III. *Fatty Acid Composition of Diacylglycerols of B. napus Leaves from Plants Grown at Different Temperatures*

Standard deviations are in parentheses, $n = 4$; tr = trace, < 0.5%.

Lipid	Growth Temperature	Fatty Acid							
		16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
	°C	mol %							
MGDG	5	1 (0)	tr	1 (0)	42 (1)	tr	1 (0)	2 (0)	53 (1)
	10	1 (0)	tr	1 (0)	41 (1)	tr	1 (1)	2 (0)	54 (1)
	20	1 (0)	tr	2 (0)	36 (2)	1 (1)	1 (0)	4 (1)	55 (1)
	30	3 (1)	tr	2 (0)	31 (1)	tr	2 (0)	5 (0)	57 (1)
DGDG	5	9 (1)	tr	1 (0)	8 (1)	1 (0)	1 (0)	3 (0)	77 (1)
	10	11 (1)	tr	1 (0)	6 (0)	1 (0)	1 (0)	4 (1)	76 (3)
	20	14 (1)	1 (0)	1 (0)	4 (1)	2 (0)	1 (0)	6 (1)	71 (2)
	30	19 (1)	tr	1 (0)	3 (0)	4 (1)	1 (0)	7 (1)	65 (1)
PC	5	18 (0)	tr	3 (0)	1 (0)	2 (0)	2 (0)	26 (1)	48 (1)
	10	18 (1)	tr	3 (0)	1 (0)	2 (1)	3 (0)	24 (1)	49 (2)
	20	19 (1)	tr	4 (1)	tr	3 (0)	7 (1)	29 (1)	38 (1)
	30	21 (1)	tr	2 (1)	tr	6 (1)	11 (1)	30 (1)	30 (1)
PG	5	32 (1)	18 (0)	3 (1)	1 (0)	2 (1)	1 (0)	8 (0)	35 (1)
	10	29 (5)	22 (1)	2 (1)	1 (1)	2 (1)	4 (3)	8 (1)	32 (1)
	20	25 (4)	25 (2)	1 (1)	1 (0)	2 (0)	5 (3)	13 (2)	28 (3)
	30	24 (4)	28 (1)	1 (0)	1 (0)	3 (1)	7 (3)	17 (2)	19 (1)
SL	5	37 (2)	tr	2 (0)	2 (0)	4 (2)	1 (0)	6 (1)	48 (3)
	10	41 (4)	tr	1 (0)	2 (1)	3 (1)	2 (1)	5 (0)	46 (3)
	20	41 (1)	tr	2 (0)	1 (0)	4 (1)	3 (1)	11 (2)	38 (3)
	30	40 (1)	tr	2 (0)	1 (0)	5 (1)	4 (1)	15 (2)	33 (1)

Table IV. *Molecular Species Composition of MGDG from B. napus Leaves Grown at Different Temperatures*

tr = trace, < 0.5%.

Molecular Species	Growth Temperature (°C)			
	5	10	20	30
	mole %			
18:3/18:3	15	16	21	22
16:3/18:3	80	74	69	61
18:3/18:2	tr	tr	tr	2
16:3/18:2	1	2	2	2
16:2/18:3	2	3	3	4
16:3/18:1	tr	1	tr	1
16:2/18:2	1	1	1	1
16:1/18:3	tr	1	tr	1
16:2/18:1	tr	tr	tr	tr
16:1/18:2	tr	tr	1	1
16:0/18:3	tr	tr	1	1
16:1/18:1	tr	tr	tr	tr
16:0/18:2	tr	tr	1	1
16:0/18:1	tr	1	tr	2

Table V. *Distribution of 16/18 and 18/18 Molecular Species of Galactosyldiacylglycerols in B. napus Leaves from Plants Grown at Different Temperatures*

Standard deviations in parentheses, $n = 4$.

Lipid	Growth Temperature	Molecular Species	
		16/18	18/18
	°C	mol %	
MGDG	5	89 (2)	11 (2)
	10	87 (2)	13 (2)
	20	81 (3)	19 (3)
	30	73 (2)	27 (2)
DGDG	5	36 (1)	64 (1)
	10	37 (3)	63 (3)
	20	41 (3)	59 (3)
	30	46 (2)	54 (2)

atures and incubated at the same temperature (20°C) to determine the effect of growth temperature on desaturation. To determine the effect of incubation temperature on the rates of desaturation, plants were grown at 10°C, and leaves were harvested and then offered $^{14}CO_2$ and incubated at 10, 20, and 30°C. A parallel experiment using plants grown at 30°C and leaves offered $^{14}CO_2$ and incubated at 30°C is shown for comparison. The results are shown for 60 min incubations for MGDG in Figure 1 and for PC in Figure 2. Similar trends were found after 30 min incubations (data not shown).

The results clearly show the rapid phase of desaturation in plants grown at 10°C. Even in plants incubated at 10°C, there was a significant degree of desaturation to 16:2 and 18:2 within 60 min. With increasing incubation temperatures (20°C and 30°C), a greater proportion of radioactivity was found in the more unsaturated fatty acids. The desaturase reactions appear to have behaved in a normal way with increasing activity at higher temperatures. Unfortunately, accurate and detailed enzyme kinetics cannot be performed on desaturase enzymes at this time. In contrast, plants grown and incubated at 30°C showed very little desaturase activity of 16C fatty acids and relatively low desaturase activity of 18C fatty acids compared to plants grown at 10°C.

Effect of Growth and Incubation Temperature on Desaturation of PC Fatty Acids. Results expressed in Table VIII indicate that the rapid phase of desaturation found in MGDG was also found in respect to desaturation of PC fatty acids. In PC, this phase is limited to 18C fatty acids as PC does not contain unsaturated 16C fatty acids. It would appear that the rapid phase in PC is less marked in comparison to 18C fatty acids of MGDG. However, Figure 2 clearly demonstrates the difference in rates of desaturation between plants grown at 10°C and incubated at different temperatures. As with MGDG fatty acid desaturation in the chloroplast, the rates of desaturation of cytosolic PC fatty acids increased with increasing temperature of incubation.

Table VI. *Distribution of Radioactivity in the Fatty Acids of MGDG after $^{14}CO_2$ Feeding and Different Periods of Incubation in $^{12}CO_2$ at 20°C*

Experiments for 0, 30, and 60 min were performed three times; experiments at 5 and 10 min were performed twice. Standard deviations are in parentheses.

Growth Temperature	Incubation Time	Percent Radioactivity in Fatty Acids							
		16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
°C	min	% dpm							
5	0	40 (3)	8 (5)	6 (0)	4 (2)	3 (2)	17 (1)	17 (0)	5 (1)
	5	28 (4)	5 (4)	13 (1)	5 (1)	1 (0)	11 (1)	27 (1)	10 (1)
	10	31 (4)	1 (1)	12 (2)	5 (0)	3 (2)	11 (2)	28 (5)	9 (2)
	30	18 (1)	2 (2)	18 (1)	11 (3)	2 (0)	6 (2)	24 (2)	19 (2)
	60	17 (1)	2 (0)	18 (0)	13 (1)	2 (0)	5 (1)	23 (1)	21 (1)
10	0	33 (10)	6 (6)	9 (3)	4 (3)	2 (1)	17 (2)	23 (1)	6 (2)
	5	23 (5)	9 (1)	13 (3)	5 (0)	2 (1)	13 (1)	26 (2)	11 (0)
	10	21 (7)	5 (5)	17 (1)	6 (1)	1 (0)	12 (0)	23 (3)	15 (2)
	30	14 (8)	3 (2)	19 (1)	11 (5)	1 (1)	6 (0)	25 (5)	21 (5)
	60	10 (7)	3 (0)	16 (4)	18 (8)	1 (1)	5 (0)	21 (6)	28 (11)
20	0	34 (5)	8 (2)	7 (1)	2 (1)	2 (1)	26 (4)	17 (3)	4 (1)
	5	28 (5)	10 (4)	7 (1)	3 (1)	1 (0)	26 (1)	19 (1)	6 (1)
	10	26 (5)	9 (2)	10 (2)	4 (0)	2 (1)	22 (5)	22 (6)	7 (1)
	30	17 (4)	6 (2)	16 (1)	8 (2)	1 (1)	13 (3)	24 (4)	15 (2)
	60	8 (2)	5 (0)	17 (2)	16 (1)	1 (1)	8 (1)	21 (1)	24 (3)
30	0	43 (1)	3 (1)	2 (1)	2 (1)	1 (1)	36 (4)	10 (3)	3 (1)
	5	40 (2)	2 (2)	3 (0)	2 (0)	1 (0)	35 (6)	14 (1)	4 (1)
	10	36 (1)	5 (2)	4 (2)	2 (0)	1 (1)	31 (3)	15 (4)	6 (1)
	30	32 (1)	6 (1)	6 (1)	3 (0)	1 (1)	25 (2)	19 (2)	8 (1)
	60	29 (9)	5 (3)	7 (4)	4 (3)	2 (0)	20 (5)	21 (1)	12 (5)

Table VII. *Effect of Age and Temperature of Growth on Incorporation of Radioactivity from $^{14}CO_2$ into Fatty Acids of MGDG*

Plants were grown at the temperatures indicated, leaves were selected according to age from first appearance of folded leaves, offered $^{14}CO_2$ for 5 min at 20°C, and extracted according to "Materials and Methods."

Growth Temperature	Leaf Age	Radioactivity in Fatty Acid							
		16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
°C	d	% dpm							
10	1	22	11	15	5		12	26	9
	5	22	9	17	5		11	27	9
	12	26	10	16	7		13	21	7
	19	28	7	17	6		10	24	8
	26	35	6	13	8		12	19	6
Mean (SD)		27 (5)	9 (2)	16 (2)	6 (1)		12 (1)	23 (3)	8 (1)
20	1	21	10	15	5		12	28	9
	5	32	6	6	1	4	30	18	3
	12	34	4	6	4	8	25	14	4
	19	38		6	3	6	26	18	4
	26	37	8	6	3	3	25	15	3
Mean (SD)		32 (6)	6 (3)	8 (4)	3 (1)	4 (3)	24 (6)	19 (5)	5 (2)
30	1	38	8	4	1	2	31	14	3
	8	41	5	2	1	2	34	12	2
	14	33	10	5	2	2	26	18	4
	19	40	7	2	1	2	35	11	2
	26	43	5	2	1	2	36	8	2
Mean (SD)		39 (3)	7 (2)	3 (1)	1 (0)	2 (0)	32 (4)	13 (3)	3 (1)

These results indicate that the rapid phase is found in at least three different desaturase reactions (16C and 18C MGDG, 18C PC) and that it varies in relative activity from one site to another.

DISCUSSION

The degree of fluidity in membranes is generally believed to be a major factor in determining cold-hardiness and tolerance of plants. Fluidity affects movement across membranes, the integrity of the membrane, and such membrane-bound processes as electron transport. A number of factors appear to affect membrane fluidity in chloroplasts, including sterol content and lipid/protein ratios. However, a predominant factor appears to be the level of desaturation of fatty acids contained in the constituent diacylglycerols of the membrane. Although many prokaryotes, animals, and plants are able to increase the levels of unsaturated fatty acids at low temperatures, no satisfactory explanation for how the organism achieves this has been advanced (8, 12). The

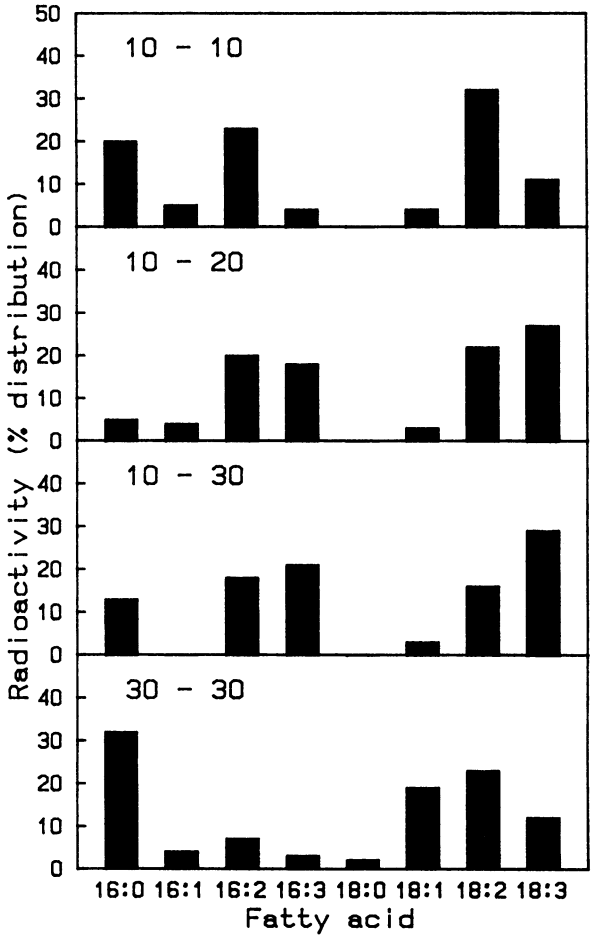

FIG. 1. Distribution of radioactivity in the major fatty acids of MGDG of leaves from *B. napus* plants grown at 10°C after $^{14}CO_2$-feeding and incubation for 60 min at 10°C (10–10), 20°C (10–20), and 30°C (10–30) and from plants grown at 30°C and incubated at 30°C (30–30).

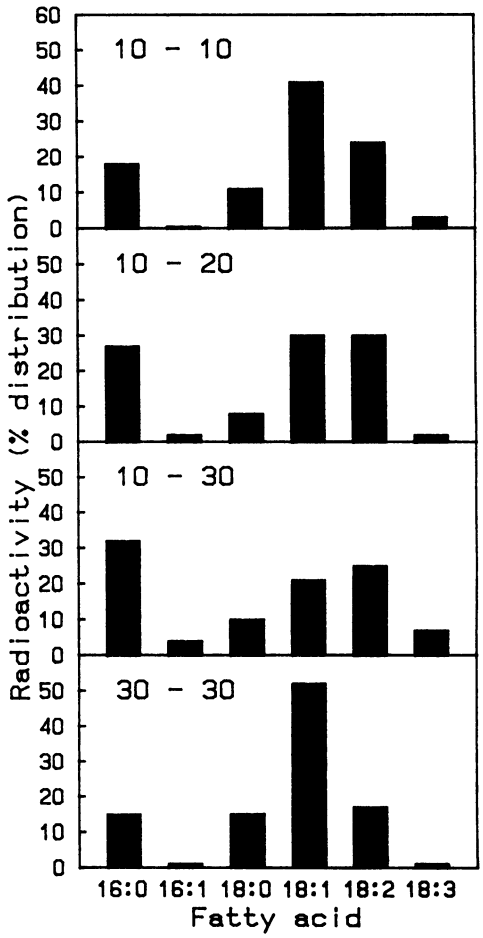

FIG. 2. Distribution of radioactivity in the major fatty acids of PC of leaves from *B. napus* plants grown at 10°C after $^{14}CO_2$-feeding and incubation for 60 min at 10°C (10–10), 20°C (10–20), and 30°C (10–30) and from plants grown at 30°C and incubated at 30°C (30–30).

fact that increased desaturation occurs at low temperatures would appear to be in contradiction to what would be expected from normal enzymic reactions.

Several theories have been advanced to explain this phenomenon in plants but none has received general support from data so far published. Our data cast further doubt on two of the major ideas proposed. Harris and James (2) proposed that increased desaturase activity is the result of increased solubility of O_2 in aqueous media at low temperatures. Our experiments, however, suggest that it is the temperature of growth not the current temperature that determines the rate of desaturation in *B. napus* leaves. In one series of experiments, we show that the rate of desaturation of leaves grown at different temperatures differs significantly from that of leaves fed and incubated at the same temperature. In a second series, we show that when plants were grown at one temperature and the leaves are fed and incubated at high or low temperatures, the rates of desaturation were highest at high temperatures as would be expected from normal enzyme reactions. These results also cast doubt on the idea of membrane fluidity determining enzyme activity and rates of desaturation in our tissue. If this were the case, it would be expected that plants grown at elevated temperatures would desaturate more rapidly when incubated at lower temperatures and vice versa in response to changes in membrane fluidity. Our data clearly indicate that plants are preconditioned by the temperature of growth and do not respond to sudden changes in environmental temperatures. They lead to the conclusion that levels of desaturation are controlled by processes such as transcription, translation, and/or post-translational modification of these enzymes.

Our results also show that when plants are grown at low temperatures there appears to exist, in addition to the normal slow process of desaturation found at higher temperatures, a second phase which results in very rapid desaturation of 16:0 to 16:2 and 18:1 to 18:2. The rates of desaturation in this phase are considerably in excess of those normally found in mature leaf tissue. Siebertz and Heinz (11) using young leaves of *Anthriscus* found comparable rates of desaturation of 16C and 18C fatty acids in MGDG. We have also found that in *Vicia faba* leaves the rate of desaturation of fatty acids in all diacylglycerols is higher in young leaves than in fully expanded, mature leaves (our unpublished data). However, in these studies with *B. napus* leaves, little difference was found between the youngest and the most mature leaves. Differences must be attributed to the growth temperature, not to the age of the leaf.

The induction of a rapid phase of desaturation at low temperatures might overcome the natural difficulty of increasing desaturase enzyme activity at these temperatures. This rapid phase could account for the higher levels of unsaturated fatty acids at lower temperatures in the lipids with which it is associated.

This rapid phase may be the result of increased or new enzyme production at low temperatures, a temperature labile modulator, or configurational changes of the membrane enzymes. The induction of enzyme reactions by cold-hardening conditions have been described by Hatano and Kabata (3) who detail the appear-

Table VIII. *Effect of Age and Temperature of Growth on Incorporation of Radioactivity from $^{14}CO_2$ into Fatty Acids of PC*

Plants were grown and extracted as described in Table VII.

Growth Temperature	Leaf Age	Radioactivity in Fatty Acid					
		16:0	16:1	18:0	18:1	18:2	18:3
°C	d	% dpm					
10	1	19	3	7	37	30	3
	5	8	5	10	41	31	4
	12	24	4	12	29	17	7
	19	23	4	9	27	27	6
	26	28	4	13	24	13	9
Mean (SD)		20 (7)	4 (1)	10 (2)	32 (6)	24 (7)	6 (2)
20	1	15	2	12	43	25	3
	5	14	1	13	54	15	2
	12	18	2	13	43	13	5
	19	22	2	20	38	12	3
	26	28	4	13	24	13	9
Mean (SD)		18 (3)	2 (1)	15 (3)	45 (5)	16 (5)	3 (1)
30	1	12	2	10	61	12	2
	8	12	2	13	58	12	2
	14	10	2	9	58	16	2
	19	11	1	11	64	12	1
	26	13	2	14	60	8	2
Mean (SD)		12 (1)	2 (0)	11 (2)	60 (2)	12 (2)	2 (0)

ance of a second fatty acid synthetase under low growth temperatures in *Chlorella*. Conformational rearrangement of enzymes in membranes in response to low temperatures might account for a more efficient biosynthesis and desaturation where and when this occurs. One way in which desaturase activity may be increased in chloroplasts, for example, might be by the concentration of galactosyl transferase and desaturase enzymes in close proximity within the envelope, ensuring more immediate access of desaturase to newly formed MGDG. If this is the case, then this might be related to development and may explain the apparently anomalous result found in 1-d-old leaves grown at 20°C (Tables VII and VIII). This would also explain why the initial reactions from 16:0 to 16:2 and 18:1 to 18:2 seem to be most enhanced in the appearance of the rapid phase.

The specific enhancement of the desaturation to 16:2 and 18:2 show that the complete desaturation to 16:3 and 18:3 in each case involves a number of separate desaturation steps and is the result of several independent reactions. However, the rapid desaturation from 16:0 through 16:1 to 16:2 suggests the possibility of a single enzyme or a multienzyme complex.

We have described the appearance of a rapid phase of desaturation in three major diacylglycerol biosynthetic reactions in plant leaves. However, our data (not shown) also indicate the presence of this rapid phase in the desaturation of 18C fatty acids in PG and SL but not in 16C desaturation to *trans*-16:1 in PG. In *B. napus* the rapid phase appears to be a general phenomenon in both cytosolic and chloroplastic diacylglycerols, although the rates of desaturation of 16C and 18C MGDG and 18C PC fatty acids have relatively different rates. This would suggest a site of action after diacylglycerol formation or the immediate DAG precursor not at the acyl coester.

The presence and variability of this rapid phase has been confirmed in other 16:3-plants (our unpublished data).

The rapid phase is responsible for the biosynthesis of 16:2(3)/18:3 molecular species of MGDG. The existence of a slower phase, which eventually results in the biosynthesis of 16:3/18:3 molecular species, also means the formation of at least a temporary pool of 16:0/18:2 (3) molecular species. This may explain the quantitative variation in diacylglycerol molecular species of different origins in MGDG and DGDG. At lower temperatures, the rapid phase results in the fast turnover of MGDG 16:0/18:1 to 16:2 (3)/18:3 species, the consequence of which is the accumulation of higher levels of 16:3/18:3 molecular species of MGDG in the chloroplast. These molecular species are not readily available for galactosylation to DGDG as indicated by the low levels of 16:3 in DGDG at all temperatures. This rapid desaturation in MGDG would lower the pool of 16/18C molecular species available to DGDG biosynthesis, particularly 16:0/18:2 (3). The end result, in agreement with our data, would be a relative decrease in 16/18C molecular species of chloroplastic origin and a relative increase in the 18/18C molecular species of cytosolic origin.

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