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Changes in the Lipid and Fatty Acid Composition of *Vicia faba* Mesophyll Protoplasts Induced by Isolation

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The lipid and fatty acid compositions of mesophyll protoplasts of Broad bean (*Vicia faba*) have been analysed by gas chromatography. The results indicate that protoplast isolation triggered the rapid hydrolysis of several membrane lipids, notably phosphatidylcholine and monogalactosyldiacylglycerol. The decrease of these lipids was compensated for by an increase in the amount of the neutral lipid fraction. Analyses of the fatty acid compositions suggest that phosphatidylcholine from the microsomes and monogalactosyldiacylglycerol from the chloroplast were degraded to diacylglycerol and free fatty acids and used for the biosynthesis of triacylglycerols.

Key words: Chloroplast — Fatty acid — Monogalactosyldiacylglycerol — Phosphatidylcholine — Protoplast — *Vicia faba*.

The lipid composition of higher plant mesophyll tissue is dominated by the choloroplast membrane lipids; MGDG, DGDG, SL, and PG. High levels of PC and PE from the extrachloroplastic membranes and trace levels of the NL, DAG, TAG, and FFA are also found in leaves.

In *Vicia faba* very high levels of the linolenic acid (18:3) are found in MGDG and DGDG while 18:2 is the predominant fatty acid in microsomal PC and PE. In addition, chloroplastic PG contains high levels of the unusual fatty acid 16:1, and significant quantities of 18:3.

In recent years, higher plant protoplasts have become very useful physiological tools (Fowke and Gamborg 1980). Despite this, the validity of protoplasts as in vivo tools has been questioned (Burgess 1978, Galun 1981). While spinach protoplasts have been used to study the subcellular localization of acetyl-CoA synthetase (Kuhn et al. 1981) and acyl carrier protein (Ohlrogge et al. 1979) they have not been used extensively to study lipid metabolism. de la Roche et al. (1977) have reported no differences in the total lipid fatty acid composition between epicotyls and their protoplasts from both hardened and unhardened winter rye and wheat. This result does not preclude, however, the possibility of changes in the fatty acid distribution within the lipid classes of protoplasts. Similarily, Haas et al. (1979) reported no significant differences in the content of lipids or total lipid fatty acids between mesophyll protoplasts and leaves of *Avena sativum*. Their data, however, suggest higher levels of DGDG in protoplasts (28 mol%) than in leaves

Abbreviations: 16:0, palmitic acid; 16:1, trans-Δ^3-hexadecenoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; DTT, dithiothreitol; FFA, free fatty acids; MGDG, monogalactosyldiacylglycerol; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SL, sulphoquinovosyldiacylglycerol; TAG, triacylglycerol; TLC, thin layer chromatography.

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(18 mol%) and elevated 18:3 levels in protoplasts (66.5%) with respect to leaves (56.4%). In view of these results it is not clear if mesophyll protoplasts are identical to their source tissue in lipid and/or fatty acid composition.

This study was undertaken to compare and characterize the lipids and fatty acid contents of leaves and mesophyll protoplasts from *Vicia faba*. A subsequent report details the metabolism of lipids and fatty acids in these protoplasts.

Materials and Methods

Plant material—Broad bean plants (*Vicia faba* var. Broad Windsor Long Pod) were grown at 20°C and 13 klux in a 16:8 h light-dark cycle. Mature leaves were harvested from 21 to 28 day-old plants at the base of the petiole and transferred to distilled water prior to protoplast isolation or lipid extraction.

Protoplast isolation—Leaves were surface sterilized by sequential rinsing for 10 s in each of 30% ethanol, water, 0.125% sodium hypochlorite, water (twice), and Murashige's minimal organic medium (pH 5.6; Flow Laboratories). Leaves were gently blotted dry and 2 g fr wt of leaf discs were transferred directly to the digestion medium. This consisted of 20 ml of Murashige's medium with 2% (w/v) Extractase PC (Fermco Biochemicals), 2% (w/v) Cellulysin (Calbiochem), 1% (w/v) ascorbic acid, 1 mM DTT, and 0.5 ml of 0.5% (w/v) $NaHCO_3$, pH 5.5. Discs were vacuum-infiltrated then allowed to stand for 1 h at 28°C and 8,000 lux with occasional shaking.

After 1 h the mixture of cells and protoplasts was separated from undigested residue by filtration through an 80 μm nylon mesh and collected by centrifugation for 5 min (50 $\times g$, 10°C; Morris and Thain 1980). The pellet was resuspended in 10 ml of digestion medium (described above) with 0.5 M mannitol. Digestion was allowed for another 30 min then protoplasts were collected by centrifugation and washed twice in 5 ml of Murashige's medium with 1% ascorbic acid, 1 mM DTT, and 0.5 M mannitol, pH 5.5.

Washed protoplast pellets were used for lipid extraction or resuspended for further incubation in 40 ml of culture medium in sterile petri plates (150 $\times$ 15 mm) under 13 klux continuous illumination at 20°C. The culture medium was adapted from those of Binding and Nehls (1978), Kao and Mickayluk (1975), and Roper (1981).

Protoplast viability was determined by Evans Blue exclusion as described by Kanai and Edwards (1973).

We consider it unlikely that we were isolating a specific population of protoplast with a lipid composition different from that of the whole leaf tissue for three reasons. First, nylon mesh filtration removed epidermis, fibres, and undigested residue. Possible vascular contamination was reduced by avoiding the midrib while cutting the leaf discs and microscopic examination showed little or no contamination from non-photosynthetic cells. Second, during cell wall digestion both columnar and spongy mesophyll cells were observed with partially digested cell walls, and were the predominant cell type present in these preparations. Finally, our recoveries were approximately 10–12% estimated on a chlorophyll basis. This recovery is expected to yield predominantly mesophyll protoplasts given that the enzymes were most accessible to palisade and spongy mesophyll cells. Furthermore, there was no difference in results obtained from samples representing a recovery as high as 25% compared to those as low as 3%. These considerations suggest that the protoplasts isolated by this method were representative samples of the original leaf tissue.

Incubation conditions—Excised leaves were maintained in distilled water side-by-side with mesophyll protoplasts in culture medium (described above). Incubation was at 13 klux con-

tinuous illumination, 20°C. After protoplast isolation samples of both leaf tissue and protoplast pellets were collected simultaneously for lipid extraction and analysis.

Lipid extraction and analysis—Leaves and protoplast pellets were extracted for lipids as described by Williams and Merrilees (1970) and the lipids separated by the TLC method of Khan and Williams (1977). In some cases the NL and pigment band from this plate was further fractionated as follows. The NL was eluted from the silica gel with chloroform–methanol (2 : 1, v/v) and chloroform. These samples were dried under N_2 then chromatographed on silica gel G plates using petroleum ether : diethyl ether : glacial acetic acid (80 : 30 : 1, v/v). Standards for TAG (corn oil) and FFA (oleic acid) were co-chromatographed with all samples.

Fatty acid methyl esters of the lipids were made with boron trifluoride (14% in methanol; Morrison and Smith 1964) then analysed by gas-liquid chromatography as described by Watson and Williams (1972).

Equipment was sterilized by autoclaving and media by filtration through 0.45 m Millipore filters. Chl was determined by the method of Arnon (1949).

Results

Protoplasts—Protoplasts isolated by this method were consistently 75–80% viable immediately after isolation as determined by the Evans Blue exclusion technique. Protoplast yields varied from $1\text{–}2 \times 10^6$ protoplasts/g fr wt of leaf material, or about 10–12% on a Chl basis.

Protoplasts were suspended in a thin layer of culture medium at a final density of approximately 4×10^5 protoplasts/ml. The half-life of these protoplasts, in culture, was determined to be about 50–55 h. In these experiments the protoplasts were in culture medium for a total of 17 h. This time is significantly shorter than the 50 h half-life to show lipid dynamics that were not simply a result of protoplast death in culture.

Lipid content—An analysis of the quantities of lipids and their component fatty acids was considered as a necessary preliminary to investigating their metabolism. Because of the nature of the total material it is not possible to determine accurately the total lipid content of cells from leaves. Isolated protoplast levels gave a mean value of 0.61 ± 0.3 moles FA/10^6 protoplasts and there was no significant change in total lipid content or loss during incubation up to 24 h. The quantities of lipid classes as mol% of the total lipid is reported in Table 1. Leaves were maintained in water as a control to indicate the effects of excision or continuous illumination on the lipid profile. Isolation of protoplasts from some of these leaves were started after 4 h of incubation and the lipids extracted from these protoplasts immediately after the end of the isolation (7 h samples) to test the effect of the isolation period on lipid and fatty acid content. Other protoplasts were maintained in liquid culture medium for a further 17 h to test for effects of culture on slower metabolic processes, and sampled at various times during incubation.

Results in Table 1 show that in control leaves the quantities of the various lipid classes were generally stable during the experiment. In contrast, there were significant differences in the lipid content between mesophyll protoplasts and the original leaf tissue. There is a marked drop in PC content from the control levels of 12–16 mol% in whole leaf tissue to 5–9 mol% in protoplasts. Similarily, there was a decrease in MGDG levels from 32–36 mol% in leaves to 16–23 mol% in protoplasts, and in DGDG levels from 22–26 mol% in leaves to 14–19 mol% in protoplasts. On the other hand, the levels of PE, SL, and PG were very similar in both leaves and protoplasts. The loss of large amounts of the membrane lipids MGDG, DGDG, and PC was accounted for by an increase in the size of the NL fraction from 7–10 mol% in control leaves to 31–40 mol% in protoplasts.

The magnitude of the decreases in PC and MGDG quantity (approximately 50%) at the end of the period required for protoplast isolation were greater than the loss in protoplast viability

Table 1 The lipid composition of *V. faba* leaves and protoplasts at various times after harvesting for protoplast isolation (See **Materials and Methods**)

Lipid	Tissue	Time (h)							
		0	2	4	7	8	9	12	24
		mol% total lipid							
PC	leaf	12(±2)	16(±4)	16(±7)	—	—	15(±5)	14(±1)	13(±2)
	protoplast	—	—	—	6(±2)	9(±1)	9(±3)	7(±2)	5(±2)
PE	leaf	7(±4)	9(±5)	5(±2)	—	—	7(±2)	5(±2)	4(±2)
	protoplast	—	—	—	2(±1)	3(±1)	5(±1)	3(±1)	4(±3)
SL	leaf	3(±2)	3(±2)	3(±1)	—	—	3(±1)	3(±1)	2(±1)
	protoplast	—	—	—	2(±1)	4(±1)	3(±1)	4(±1)	7(±7)
DGDG	leaf	26(±2)	23(±2)	23(±1)	—	—	23(±3)	22(±3)	25(±3)
	protoplast	—	—	—	17(±1)	17(±2)	14(±5)	16(±4)	19(±6)
PG	leaf	12(±2)	12(+2)	11(±0)	—	—	11(±1)	10(±1)	10(±1)
	protoplast	—	—	—	11(±2)	15(±0)	15(±6)	12(±2)	9(±5)
MGDG	leaf	35(±4)	32(±8)	34(±4)	—	—	34(±3)	36(±2)	35(±2)
	protoplast	—	—	—	23(±3)	21(±4)	19(±2)	20(±3)	16(±3)
NL	leaf	7(±2)	7(±1)	8(±2)	—	—	7(±2)	10(±3)	9(±2)
	protoplast	—	—	—	39(±2)	31(±5)	37(±11)	39(±7)	40(±14)

Protoplast isolation was started at 4 h and was completed in time for analysis at 7 h. Values are means of 2–5 separate experiments.

at that time (20–25%), indicating that changes in lipid content occurred rapidly in intact and viable mesophyll protoplasts.

It is notable that the bulk of the observed changes of lipid content occurred during the

Table 2 The fatty acid composition of PC from *V. faba* leaves and protoplasts at various times after harvesting for protoplast isolation (see **Materials and Methods**)

Fatty acid	Tissue	Time (h)							
		0	2	4	7	8	9	12	24
		mol% total PC fatty acids							
16:0	leaf	19(±1)	18(±2)	17(±4)	—	—	18(±2)	19(±1)	19(±1)
	protoplast	—	—	—	22(±0)	21(±2)	22(±5)	23(±1)	28(±5)
18:0	leaf	4(±1)	3(±0)	4(±1)	—	—	4(±0)	5(±0)	6(±1)
	protoplast	—	—	—	6(±1)	5(±2)	6(±1)	6(±1)	6(±2)
18:1	leaf	20(±4)	21(±3)	22(±4)	—	—	26(±8)	22(±4)	14(±3)
	protoplast	—	—	—	20(±1)	21(±5)	16(±6)	19(±2)	17(±3)
18:2	leaf	41(±4)	41(±2)	37(±3)	—	—	35(±5)	39(±1)	41(±6)
	protoplast	—	—	—	27(±1)	27(±0)	28(±3)	24(±1)	21(±3)
18:3	leaf	16(±2)	17(±2)	20(±11)	—	—	17(±6)	16(±1)	20(±2)
	protoplast	—	—	—	26(±3)	28(±1)	28(±2)	28(±2)	28(±3)

Protoplast isolation was started at 4 h. Values are means of 2–5 separate experiments.

Table 3 The fatty acid composition of PE from *V. faba* leaves and protoplasts at various times after harvesting for protoplast isolation (see **Materials and Methods**)

Fatty acid	Tissue	Time (h)							
		0	2	4	7	8	9	12	24
		mol% total PE fatty acids							
16:0	leaf	26(±4)	28(±5)	31(±4)	—	—	30(±4)	29(±2)	29(±4)
	protoplast	—	—	—	31(±6)	24(±2)	30(±4)	32(±5)	36(±3)
18:0	leaf	5(±3)	4(±1)	4(±1)	—	—	4(±0)	4(±1)	5(±1)
	protoplast	—	—	—	10(±2)	8(±0)	11(±5)	9(±3)	13(±4)
18:1	leaf	9(±1)	10(±1)	9(±2)	—	—	11(±3)	10(±2)	8(±2)
	protoplast	—	—	—	8(±2)	15(±3)	7(±4)	11(±5)	9(±3)
18:2	leaf	46(±5)	45(±4)	41(±2)	—	—	41(±3)	42(±3)	42(±7)
	protoplast	—	—	—	25(±4)	24(±3)	26(±9)	20(±2)	16(±6)
18:3	leaf	13(±4)	14(±2)	15(±2)	—	—	15(±3)	15(±4)	16(±2)
	protoplast	—	—	—	26(±2)	29(±3)	26(±7)	28(±4)	26(±2)

Protoplast isolation was started at 4 h. Values are means of 2–5 separate experiments.

3–4 h period required for protoplast isolation but levels were relatively stable during the following 17 h of maintenance in culture medium.

Fatty acid content—The fatty acid content of PC in leaves and protoplasts is shown in Table 2. The fatty acid profile of PC from whole control leaves was stable during the experiment. Isolation of mesophyll protoplasts, however, induced significant changes in the fatty acid content of PC. In protoplasts the 18:2 level dropped sharply during the isolation period from approximately 40 mol% to 26 mol% while the 18:3 proportion increased from 16 mol% to 28 mol%.

Table 4 The fatty acid content of PG from *V. faba* leaves and protoplasts at various times after harvesting for protoplast isolation (see **Materials and Methods**)

Fatty acid	Tissue	Time (h)							
		0	2	4	7	8	9	12	24
		mol% total PG fatty acids							
16:0	leaf	19(±5)	21(±2)	22(±1)	—	—	23(±0)	22(±1)	22(±2)
	protoplast	—	—	—	21(±0)	29(±3)	23(±2)	23(±3)	29(±5)
16:1	leaf	31(±2)	29(±3)	27(±3)	—	—	29(±2)	27(±2)	27(±2)
	protoplast	—	—	—	28(±0)	24(±0)	25(±2)	27(±4)	20(±5)
18:0	leaf	3(±1)	2(±1)	3(±1)	—	—	2(±0)	3(±1)	3(±1)
	protoplast	—	—	—	4(±1)	3(±0)	4(±1)	4(±1)	6(±2)
18:1	leaf	5(±1)	5(±1)	6(±0)	—	—	5(±1)	6(±1)	6(±0)
	protoplast	—	—	—	6(±1)	9(±1)	7(±1)	6(±1)	6(±1)
18:2	leaf	15(±2)	16(±3)	16(±4)	—	—	14(±1)	17(±2)	17(±1)
	protoplast	—	—	—	13(±1)	11(±0)	14(±2)	14(±1)	12(±2)
18:3	leaf	28(±3)	27(±4)	27(±5)	—	—	26(±3)	26(±3)	25(±2)
	protoplast	—	—	—	27(±0)	25(±1)	28(±3)	28(±3)	27(±2)

Protoplast isolation was started at 4 h. Values are means of 2–5 separate experiments.

Table 5 The fatty acid composition of MGDG from *V. faba* leaves and protoplasts at various times after harvesting for protoplast isolation (see **Materials and Methods**)

Fatty acid	Tissue	Time (h)							
		0	2	4	7	8	9	12	24
		mol% total MGDG fatty acids							
16:0	leaf	3(±1)	2(±0)	2(±1)	—	—	2(±0)	2(±0)	2(±0)
	protoplast	—	—	—	5(±2)	3(±1)	5(±2)	5(±1)	8(±4)
16:1	leaf	n.d.	n.d.	n.d.	—	—	n.d.	n.d.	n.d.
	protoplast	—	—	—	2(±1)	n.d.	1(±1)	2(±2)	2(±2)
18:0	leaf	1(±0)	1(±1)	1(±0)	—	—	1(±0)	1(±0)	1(±0)
	protoplast	—	—	—	2(±0)	1(±0)	2(±2)	1(±1)	2(±1)
18:1	leaf	1(±1)	1(±1)	1(±1)	—	—	1(±0)	1(±0)	1(±0)
	protoplast	—	—	—	2(±1)	5(±0)	2(±1)	2(±1)	3(±1)
18:2	leaf	5(±1)	5(±0)	5(±1)	—	—	5(±1)	6(±1)	7(±1)
	protoplast	—	—	—	7(±1)	6(±1)	7(±2)	7(±1)	9(±2)
18:3	leaf	90(±1)	91(±1)	92(±1)	—	—	91(±1)	90(±1)	90(±1)
	protoplast	—	—	—	82(±5)	85(±1)	82(±5)	83(±3)	81(±2)

n.d.: not detected

Protoplast isolation was started at 4 h. Values are means of 2–5 separate experiments.

In this respect the fatty acid composition of protoplast PC was more similar to that of chloroplast PC, in which the ratio of 18:3/18:2 is higher than in microsomal fractions (Williams et al. 1979). Similar data were obtained for the fatty acid composition of PE (Table 3). For both lipids the

Table 6 The fatty acid composition of the NL fraction from *V. faba* leaves and protoplasts at various times after harvesting for protoplast isolation (see **Materials and Methods**)

Fatty acid	Tissue	Time (h)							
		0	2	4	7	8	9	12	24
		mol% total NL fatty acids							
16:0	leaf	36(±8)	34(±5)	30(±4)	—	—	30(±5)	30(±7)	29(±10)
	protoplast	—	—	—	16(±2)	19(±2)	20(±6)	20(±3)	20(±6)
cis-16:1	leaf	13(±0)	3(±1)	6(±1)	—	—	4(±0)	2(±2)	n.d.
	protoplast	—	—	—	1(±0)	7(±2)	4(±3)	2(±1)	2(±1)
18:0	leaf	8(±4)	10(±3)	8(±2)	—	—	9(±4)	11(±4)	6(±1)
	protoplast	—	—	—	5(±4)	8(±0)	10(±4)	7(±3)	8(±3)
18:1	leaf	11(±4)	9(±2)	8(±4)	—	—	10(±1)	12(±8)	7(±1)
	protoplast	—	—	—	6(±2)	13(±3)	9(±4)	9(±3)	11(±3)
18:2	leaf	18(±1)	17(±4)	16(±3)	—	—	18(±3)	19(±3)	21(±6)
	protoplast	—	—	—	12(±2)	12(±2)	12(±3)	13(±2)	12(±3)
18:3	leaf	27(±10)	30(±5)	31(±6)	—	—	31(±3)	26(±10)	37(±7)
	protoplast	—	—	—	59(±10)	41(±0)	47(±13)	50(±10)	46(±10)

n.d.: not detected

Protoplast isolation was started at 4 h. Values are means of 2–5 separate experiments.

Table 7 The mol% ratio of 18:2/18:3 in the TAG from leaves and protoplats of *V. faba* at various times after harvesting for protoplast isolation (see **Materials and Methods**)

Lipid	Tissue	Time (h)						
		0	2	4	8	9	12	24
TAG	leaf	1.1	1.7	1.3	—	1.2	1.6	1.1
	protoplast (a)	—	—	—	0.1	0.5	0.4	0.4
	protoplast (b)	—	—	—	0.4	0.6	0.4	0.5

Protoplast isolation was started at 4 h. Values are from a single experiment with replicates (a and b) of the protoplast samples.

major changes occurred during the isolation period while the fatty acids were stable when occurred during the isolation period while the fatty acids wree stable when protoplasts were maintained in culture medium.

The fatty acid composition of PG is given in Table 4. In contrast to the other phospholipids, PG showed little change in the fatty acid profile at the end of protoplast isolation (Table 4, 7 h).

The fatty acid composition of MGDG is shown in Table 5. In whole leaves the 18:3 level was 90 mol% or more of the total MGDG fatty acids while 16:0 and 18:2 made up the bulk of the remainder. In protoplasts the 18:3 content decreased to 82 mol% after protoplast isolation and remained stable during maintenance in culture medium. The decreased 18:3 quantity was matched by a 2–3 fold increase in 16:0 levels while 18:2 increased only marginally. One of the explanations for this disproportionate increase in 16:0 in MGDG is that continued synthesis of MGDG from a precursor DAG containing high levels of 16:0 occurred in these protoplasts. Supporting evidence for this is provided in a subsequent report (Webb and Williams 1984). The fatty acid compositions of DGDG and SL (data not shown) showed no significant changes in the fatty acid profile of either lipid despite significant loss in the total quantity of DGDG. This lack of a preferential loss of the polyunsaturated fatty acids in DGDG and SL suggests that photooxidation of fatty acids in lysed protoplasts had a negligible contribution to the fatty acid changes reported here.

The fatty acid composition of the NL fraction is presented in Table 6. The quantity of 18:3 in the NL increased rapidly from approximately 30 mol% in leaves to 40–60 mol% in protoplasts and is compensated for by decreases of both the 16:0 and 18:2 levels. The decrease in 16:0 suggests that the higher 18:3 quantities did not result from an initiation of 18:2 desaturation in the protoplast NL. Rather, this 18:3 may be contributed by degradation of MGDG. Further analysis showed that the 18:3 increase was confined mostly to the TAG portion of the NL fraction. This is shown in Table 7 in which the 18:2/18:3 ratio in the TAG decreased from greater than one in leaves to approximately 0.5 in protoplasts. The fatty acid compositions of both DAG and FFA were stable in leaves and protoplasts with the major fatty acids being 16:0, 18:0, and 18:1 with greatly reduced levels of 18:2 and 18:3 (data not shown).

Discussion

The lipid and fatty acid composition of *Vicia faba* mesophyll protoplasts has been shown to be significantly different from that of the original leaf tissue. Further, the data suggest that these changes occurred rapidly and almost exclusively during the 3 to 4 h period required for protoplast isolation. Lipid and fatty acid profiles were found to be relatively stable in protoplasts main-

tained in culture medium for up to 17 h. While it might be argued that we have simply isolated a subpopulation of cells of different lipid and fatty acid composition, our yield of 10–12% Chl and microscopic observations suggested that the protoplasts were derived predominantly from spongy and palisade mesophyll cells.

If these protoplasts are representative samples of the original leaf tissue, then rapid changes in the lipid and fatty acid compositions occurred during the 3–4 h period required for protoplast isolation. Polar lipids were degraded, probably through acylhydrolase or acyltransferase activity (Galliard 1980) and the resultant DAG and FFA used for the synthesis of TAG. If so, then the quantity of total lipid should be equal in leaves and protoplasts. Analysis of the total lipid content gave higher levels in protoplasts ($16.9+8.7$ mol fatty acid/mol Chl, $n=19$) than in leaves (11.0 ± 4.9 mol fatty acid/mol Chl, $n=24$), however, it is reasonable to suppose that chlorophyll levels decreased in these protoplasts and accounted for this elevated ratio in protoplasts. The stability of the polyunsaturated fatty acids with respect to saturated fatty acids in both DGDG and SL suggests that significant photo-oxidation did not occur. We conclude, therefore, that there is no evidence for net destruction of fatty acid residues in these preparations.

These data indicate some selectivity in the loss of membrane lipids from both extrachloroplastic and chloroplastic compartments of protoplasts. In the former, both PC and PE decreased (Table 1) and the change in the total PC fatty acid composition (Table 2) to one similar to that of chloroplast PC (Williams et al. 1979) indicates that microsomal, but not chloroplastic, PC was degraded. This chloroplastic PC may well be located in the envelope. Furthermore, this implies that chloroplastic and microsomal hydrolytic activities have different specificities since the microsomal activity hydrolyzed PC whereas the chloroplastic activity acted on MGDG and DGDG but apparently had little or no activity towards either PC or PG.

The nature of this degradation is not clear. The appearance of fatty acids from degraded polar lipids in NL fraction (Table 1) implies that these lipids were attacked by a non-specific lipid acylhydrolase (Galliard 1980). A fraction has been isolated from leaves of *Phaseolus multiflorus* (Burns et al. 1977a, b) that released FFA from MGDG, DGDG, PC, and SL but showed little activity against tristearoylglycerol (Burns et al. 1977a). This fraction reacted with highly unsaturated molecular species of SL rather than molecular species containing 16:0 (Burns et al. 1977b). We did not, however, detect significant changes in SL levels in these protoplasts. Instead, chloroplast lipid hydrolysis may be due to an enzyme related to the galactolipid lipase isolated from *Phaseolus vulgaris* chloroplasts (Anderson et al. 1974) which attacks subchloroplastic particles, MGDG dispersed with Triton x-100, dipalmityl-PC, and DGDG, but not TAG.

These results show that the accumulation of NL occurred very quickly after the start of protoplast isolation. This is consistent with the results of Gigot et al. (1975) and Taylor and Hall (1978) who have reported the appearance of large osmiophilic droplets in the chloroplasts and cytoplasm of tobacco mesophyll protoplasts. These droplets were often closely associated with both cytoplasmic membranes and the chloroplast envelope and were probably lipidic in nature (Taylor and Hall 1978). It is reasonable to suggest that an isolation-association stress triggered the hydrolysis of polar lipids and the corresponding synthesis of NL. In this respect, protoplasts showed lipid metabolism that was qualitively similar to that reported for senescent leaves (Barton 1966) and leaf discs (Newman et al. 1973).

It is not clear from these results whether decreases in the quantities of some of the polar lipids were due solely to elevated catabolic activities and/or due to decreased or inhibited biosynthetic processes. As subsequent report will investigate the turnover of the lipids and fatty acids of *Vicia faba* mesophyll protoplasts (Webb and Williams 1984).

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