The role of phosphatidylcholine in fatty acid exchange and desaturation in *Brassica napus* L. leaves

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The role of phosphatidylcholine (PC) in fatty acid exchange and desaturation was examined and compared with that of monogalactosyldiacylglycerol (MGDG) in *Brassica napus* leaves using ^{14}C-labelling *in vivo*. Data are presented which indicate that in the chloroplast newly formed saturated (palmitic acid, 16:0) and monounsaturated (oleic acid, 18:1) fatty acid is incorporated into MGDG and desaturated *in situ*. In the non-plastidic compartments, however, newly formed fatty acid is exchanged with polyunsaturated fatty acid in PC, the probable major site of subsequent desaturation. The unsaturated fatty acid is released to the acyl-CoA pool, which is then used to synthesize diacylglycerol (DAG) containing a high level of unsaturated fatty acid. This highly unsaturated DAG may be the source for the biosynthesis of other cellular glycerolipids. The generally accepted pathway in which PC is synthesized from molecular species of DAG containing 16:0 and 18:1 followed by desaturation of the 18:1 to linoleic (18:2) and linolenic (18:3) acids is questioned.

Key words: acyl-CoA, ^{14}C-labelling, lipid molecular species, plant lipid.

INTRODUCTION

Phosphatidylcholine (PC) is the major phospholipid in most higher plant cells and is found in most non-plastidic membranes, endoplasmic reticulum, mitochondria, plasma membrane and tonoplast, and the outer-envelope membrane of the chloroplast. It occupies a key position in membrane structure and glycerolipid biosynthesis in leaves and the biosynthesis of triacylglycerol (TAG) in seeds of oilseed plants [1]. While it is located primarily in the non-plastidic membranes, in most plants it is believed to be a major site of fatty acid desaturation and a precursor of chloroplastic lipids in leaves [2,3]. In oilseeds PC appears to play a similar role, being largely responsible for the levels of unsaturated fatty acids in seed-oil TAGs [4]. Despite its importance in leaf chloroplast lipid and seed-oil biosynthesis, surprisingly little is known about the details of the synthesis and desaturation of fatty acids in association with PC and their transfer to the chloroplast.

There is considerable evidence that palmitoyl- and oleoyl-acyl carrier protein are the immediate products of fatty acid synthase and stearoyl-acyl carrier protein desaturase in plastids [5]. These acyl chains are exported from the plastid as free fatty acids, which are then esterified to CoA in the cytosol. The acyl-CoA thioesters exist as a soluble pool in the cytosol from which they are used in the biosynthesis of diacylglycerol (DAG) and subsequently phospholipids and TAG [1]. It is generally assumed that PC is formed from DAG containing mainly oleic acid (18:1; esterified to the *sn*-1 and -2 positions) and palmitic acid (16:0; on the *sn*-1 position) and that subsequent desaturation of the 18:1 *in situ* results in the production of linoleic (18:2) and linolenic (18:3) acids [1]. In leaves, PC containing highly unsaturated fatty acid may then be used as a structural component of cytoplasmic membranes or as a precursor to the DAG used in the production of other cytoplasmic phospholipids (e.g. phosphatidylethanolamine) and chloroplast lipids [monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulphoquinovosyldiacylglycerol (SQDG)]. In seeds a similar pathway is hypothesized except that there is some exchange of fatty acid between PC and the acyl-CoA prior to DAG and TAG synthesis [6,7]. The desaturation of fatty acids in PC determines the levels of unsaturated fatty acids in the DAG precursors of seed TAG and the chloroplast lipids [8]. Despite this assumed role in chloroplast lipid biosynthesis no confirmed pathway for the transfer of the DAG moiety from PC to the chloroplast has ever been described. Recently Mongrand et al. [9] have challenged this hypothesis and proposed that the DAG from PC may not be integrated intact into the chloroplast lipids.

It has been shown that the biosynthesis of MGDG, DGDG and SQDG in chloroplasts occurs predominantly within the chloroplast envelope from DAG derived either from the chloroplast envelope itself (prokaryotic or chloroplastic pathway) or from a source outside the chloroplast (eukaryotic pathway) [1]. If the source of the DAG is from within the chloroplast, the DAG normally contains 18:1 (on the *sn*-1 position) and 16:0 (on the *sn*-2 position), a typical prokaryotic structure. If the source of the DAG is from outside the chloroplast, the molecular species of MGDG, DGDG and SQDG formed are of the eukaryotic structure, normally containing a mixture of 18:2 and 18:3 at both *sn* positions and 16:0 on the *sn*-1 position. Subsequent desaturation *in situ* of fatty acids in prokaryotic species [in hexadecatrienoic acid (16:3) plants] results in the production of mainly 18:3/16:3 molecular species of MGDG and DGDG, and 18:3/16:0 molecular species of SQDG [10]. Fatty acid desaturation in eukaryotic species results in predominantly 18:3/18:3 molecular species in MGDG and DGDG and 16:0/18:3 and 18:3/18:3 species in SQDG [10].

It is generally believed that desaturation of fatty acids on PC and MGDG in the chloroplast occurs *in situ*. Evidence for this is

Abbreviations used: DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; lyso-PC, lysophosphatidylcholine; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; SQDG, sulphoquinovosyldiacylglycerol; TAG, triacylglycerol; 16:0, palmitic acid; 16:2, hexadecadienoic acid; 16:3, hexadecatrienoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 18:4, octadecatetraenoic acid.

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derived mainly from ^{14}C-labelling experiments *in vivo* and *in vitro* [1,5]. These data have been interpreted to suggest that PC and MGDG are formed from mono- and diene molecular species [18:1(16:0)/18:1 and 18:1/16:0, respectively] and that further desaturation occurs on the lipid to form the highly unsaturated fatty acids typical of PC and MGDG in different species. In 16:3 plants, only part of the DAG backbone of chloroplast lipids is thought to be derived from PC, whereas in 18:3 and octadecatetraenoic acid (18:4) plants all the DAG backbones of MGDG, DGDG and SQDG are presumed to be derived from PC [11]. In all plants it is believed that the intact lipid is the substrate for desaturases in the prokaryotic and the eukaryotic pathways [1,5]. However, evidence has been presented that suggests that the acyl-CoA esters (see [5]) may also be substrates for desaturases.

We undertook the experiments reported here to show in more detail the distribution of newly formed fatty acids on the molecular species of PC and MGDG with the objective of comparing the two biosynthetic pathways. While it is generally assumed that they are similar, our data show very significant differences between these pathways, suggesting that the role PC plays in fatty acid desaturation and the origin of the eukaryotic DAG in chloroplast lipid biosynthesis need to be re-examined.

Table 1 Fatty acid composition and distribution of radioactivity in MGDG and PC after ^{14}C-labelling of leaf discs and incubation for 1, 24 and 48 h in continuous light

Data are means ± S.D. obtained from three different experiments. 16:1, palmitoleic acid; 16:2, hexadecadienoic acid; 18:0, stearic acid.

	Incubation time ...	1 h		24 h		48 h	
Lipid	Fatty acid	mol%	% d.p.m.	mol%	% d.p.m.	mol%	% d.p.m.
MGDG	16:0	3±2	25±1	3±3	11±2	3±1	3±1
	16:1	1±1	2±2	1±1	1±1	0±0	1±0
	16:2	4±1	6±1	3±1	7±1	4±1	4±0
	16:3	30±1	4±2	33±1	18±1	31±1	30±1
	18:0	1±1	2±2	1±1	1±2	1±0	1±0
	18:1	1±1	27±2	1±0	4±1	1±0	2±2
	18:2	8±2	22±1	5±1	18±1	7±2	6±2
	18:3	54±0	8±1	55±1	38±2	51±3	50±2
PC	16:0	18±1	14±2	19±2	13±1	18±1	18±1
	18:0	4±1	6±1	4±1	4±1	3±1	3±0
	18:1	3±1	47±2	6±2	21±2	5±2	13±1
	18:2	30±2	19±2	31±2	43±1	32±1	47±2
	18:3	42±2	7±1	42±1	12±1	39±1	17±2

EXPERIMENTAL

Brassica napus plants were grown from seed in growth rooms at 20 °C under a 16-h:8-h light/dark regime with mixed fluorescent/incandescent light (approx. 200 $\mu E \cdot s^{-1} \cdot m^{-2}$) for 3–4 weeks. Mature leaves were sampled, cut into discs (1.7 cm in diameter) and the discs were fed $^{14}CO_2$ in light for 5 min. A 2-g sample of the discs was taken immediately for lipid extraction (0 h) and the remaining discs were incubated under the same light intensity and temperature for periods up to 48 h in continuous light. Samples of 2 g of discs were taken at intervals during this incubation period.

The leaf discs were extracted with chloroform/methanol (2:1, v/v) and the lipid extracts purified as described previously [12]. Lipid classes were separated by TLC and molecular species of PC and MGDG were separated by argentation TLC as described previously [13]. The fatty acids of each molecular species band from the TLC plates were methanolysed using sodium methoxide [13] and the subsequent fatty acid methyl esters were analysed quantitatively using a Hewlett-Packard model 5890 gas-liquid chromatograph on a 30-m DB-23 capillary column programmed from 160 to 200 °C at 3 °C·min^{-1}. A portion of the sample was separated in a Hewlett-Packard model 7300 gas-liquid chromatograph on a packed 1.8 m × 4 mm glass column using Reoplex 400 as stationary phase with an isothermal column temperature of 180 °C. Each fatty acid was collected and radioactivity measured by scintillation counting as described previously [14]. Each analysis was completed on at least three different samples. However, because the uptake of label from $^{14}CO_2$ is variable between experiments, it is not possible to average samples except as percentage distributions. The data presented for the quantitative distribution and radioactivity of molecular species are, therefore, results from one experiment that was typical of at least three similar analyses.

RESULTS

^{14}C Labelling of MGDG and PC

B. napus leaf discs analysed after $^{14}CO_2$ feeding showed the typical fatty acid profile of MGDG and PC in 16:3 plants with little change in the fatty acid composition during the incubation periods up to 48 h (Table 1).

In MGDG 1 h after $^{14}CO_2$ labelling, radioactivity was found predominantly and almost equally in 16:0 and 18:1. This is consistent with the hypothesis that the initial labelling of MGDG is through the prokaryotic pathway and that the species formed is 18:1/16:0. Even at 1 h, however, there were significant levels of radioactivity in hexadecadienoic acid (16:2), 18:2 and 18:3, indicating an initial rapid desaturation phase as described previously [15]. After 24 and 48 h, progressively more of the radioactivity was converted more slowly by desaturation to 16:2 and 16:3, and 18:2 and 18:3, until after 48 h the distribution of label was similar to the mol% distribution of fatty acids (Table 1).

In PC 1 h after labelling, the majority of radioactivity was found in 18:1 and lesser amounts in 16:0, 18:2 and 18:3 (Table 1). This suggests the synthesis of PC from 18:1(16:0)/18:1 molecular species and a rapid desaturation of part of the 18:1 to 18:2 and 18:3 [15]. In contrast to MGDG, however, after 24 and 48 h incubation there is only a small increase in label in 18:3 and the levels of radioactivity are significantly lower than the mol% distribution.

Molecular species of MGDG and PC

Detailed analyses indicate that in MGDG there are two predominant molecular species (18:3/18:3 and 18:3/16:3) formed through the eukaryotic and prokaryotic pathways, respectively (Figure 1A). Pentaene and tetraene molecular species make up the bulk of the remaining molecular species. The most highly labelled molecular species after $^{14}CO_2$ feeding (18:1/16:0; see below) is found in less than 1% of the total MGDG, suggesting a transient role in the biosynthetic pathway.

The separation of molecular species of PC indicates that there are more major molecular species components (18:3/18:3, 18:3/18:2, 18:2/18:2, 16:0/18:3 and 16:0/18:2; Figure 1B) than in MGDG. The assumed initial product of biosynthesis, 18:1(16:0)/18:1 molecular species of PC, occurs in very low concentration. The major difference between MGDG and PC is that in PC there is less complete desaturation of the fatty acids and a greater array of different molecular species. This is also

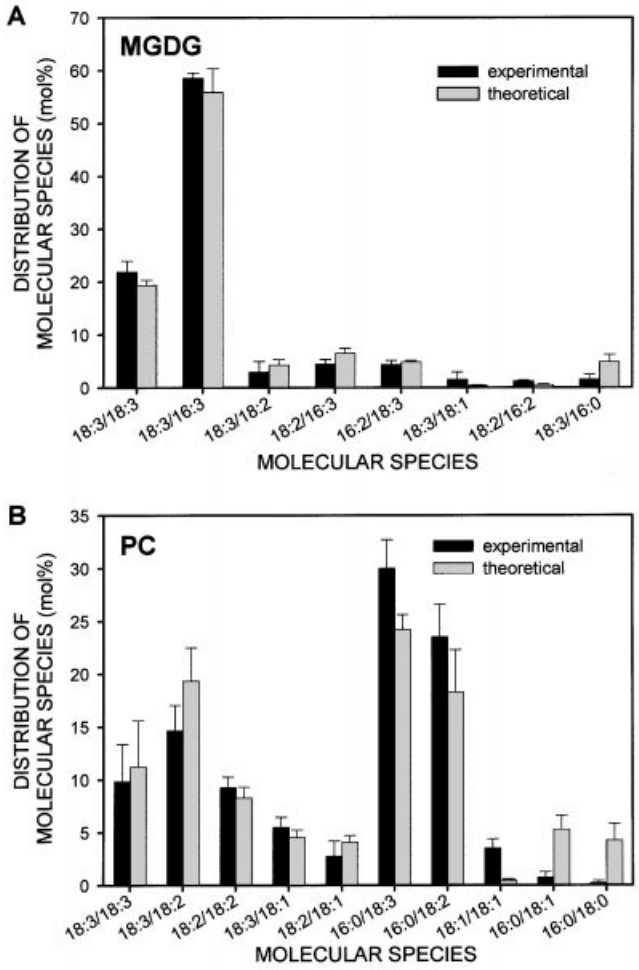


Figure 1 Distribution of molecular species of MGDG and PC determined experimentally by argentation TLC and GLC, and theoretically from the fatty acid composition of the total lipid samples analysed using a computer program to determine the theoretical distribution

The program assumes the random distribution of the fatty acids except for 16C on the *sn*-2 position of MGDG and 16:0 on the *sn*-1 position of PC. The data are from labelling experiments that had been incubated for different periods up to 6 h after ^{14}C feeding ($n = 5$, $\pm$S.D.). Only molecular species that are greater than 1% (either theoretical or experimental determinations) are represented.

consistent with the other major lipids in the cell; the chloroplast lipids (MGDG, DGDG and SQDG) show very high levels of unsaturation and the non-chloroplastic lipids (PC, phosphatidylethanolamine) show lower, incomplete desaturation (results not shown).

Using a computer program it is possible to calculate the theoretical random distribution of molecular species from the total fatty acid composition of a DAG, assuming that all C_{16} fatty acids in MGDG are found on the *sn*-2 position and 16:0 in PC is confined to the *sn*-1 position. A comparison of the experimentally determined levels of molecular species with these theoretical random distributions shows that both MGDG and PC have close to random distribution of the fatty acid in molecular species (Figure 1). In PC, the 16:0/18:1 and 16:0/stearic acid (18:0) levels appear to be significantly below the theoretical levels. This probably results in the levels of other molecular species containing 16:0 or 18:0 (16:0/18:3, 16:0/18:2 and 18:0/18:2) being higher than the theoretical values. This suggests that some factor militates against the biosynthesis or stability of molecular species containing 0 or only 1 unsaturated bond.

^{14}C Labelling of fatty acids in molecular species of MGDG

Because of the almost equal labelling of 18:1 and 16:0 in MGDG at early feeding times it is assumed that initial synthesis occurs by the galactosylation of DAG containing 18:1 on the *sn*-1 position and 16:0 on the *sn*-2 position in the prokaryotic pathway. Analyses of the radioactive content of the fatty acids of MGDG molecular species after short-term (1 h) feeding confirm this (Figure 2A). After 1 h of incubation most of the radioactivity was found in the 18:1/16:0 molecular species with almost equal levels in each of the two fatty acids. In molecular species in which some desaturation had occurred, the levels of radioactivity in each of the two fatty acids was almost equal, even in molecular species (e.g. 18:3/16:2 and 18:2/16:3) in which unequal desaturation had occurred at the two *sn* positions. After 24 h (Figure 2B) and 48 h (results not shown), a similar pattern was found except that at these times significant desaturation had occurred and most of the radioactivity was now located in the more unsaturated species. In all cases, however, the levels of radioactivity in the two fatty acids in each molecular species were similar.

In our view these data are completely consistent with the hypothesis that: (i) the majority of MGDG is synthesized through the prokaryotic pathway from 18:1/16:0 DAG, (ii) the fatty acids are desaturated *in situ* on MGDG to 18:3/16:3, and (iii) there is little or no exchange of fatty acid between molecular species.

^{14}C Labelling of fatty acids in molecular species of PC

It has long been accepted that fatty acids in PC are desaturated *in situ* after the initial synthesis of PC from 18:1/18:1 and 16:0/18:1 DAG [1]. If this is the case a similar labelling pattern to that found in MGDG would be expected in PC. In other words, if PC is formed from 18:1/18:1 and 16:0/18:1 molecular species, these are the molecular species where most of the initial radioactivity would be located and the two fatty acids of all molecular species after subsequent desaturation would be similarly labelled, as shown in MGDG above.

The analyses of PC molecular species (Figure 3) show the opposite results to those found in MGDG and are, therefore, not consistent with the hypothesis that PC is formed from 18:1/18:1 and 16:0/18:1 molecular species of DAG. Most of the radioactivity was located in 18:1 1 h after ^{14}C labelling (Table 1); however, the radioactive 18:1 was not confined to 18:1/18:1 but distributed between the molecular species containing 18:1 (18:1/18:1, 18:1/18:2, 18:1/18:3; Figure 3A). Furthermore, in heterogeneous molecular species the accompanying fatty acids (18:2, 18:3) contain significantly less radioactivity and, therefore, could not be formed by desaturation of 18:1 during incubation after ^{14}C feeding. Similarly, the label found in 16:0 in the 16:0/18:3 molecular species is higher than in 18:3, and the label in 18:2 is higher than 18:3 in the 18:2/18:3 molecular species.

After 24 h of incubation, the 18:1 has been largely desaturated to 18:2 (Table 1). In molecular species that contain 18:1 the radioactive labelling pattern is similar to that found at 1 h except that the levels of radioactivity in 18:2 and 18:3 are higher (Figure 3B). In other molecular species containing 18:2 (par-

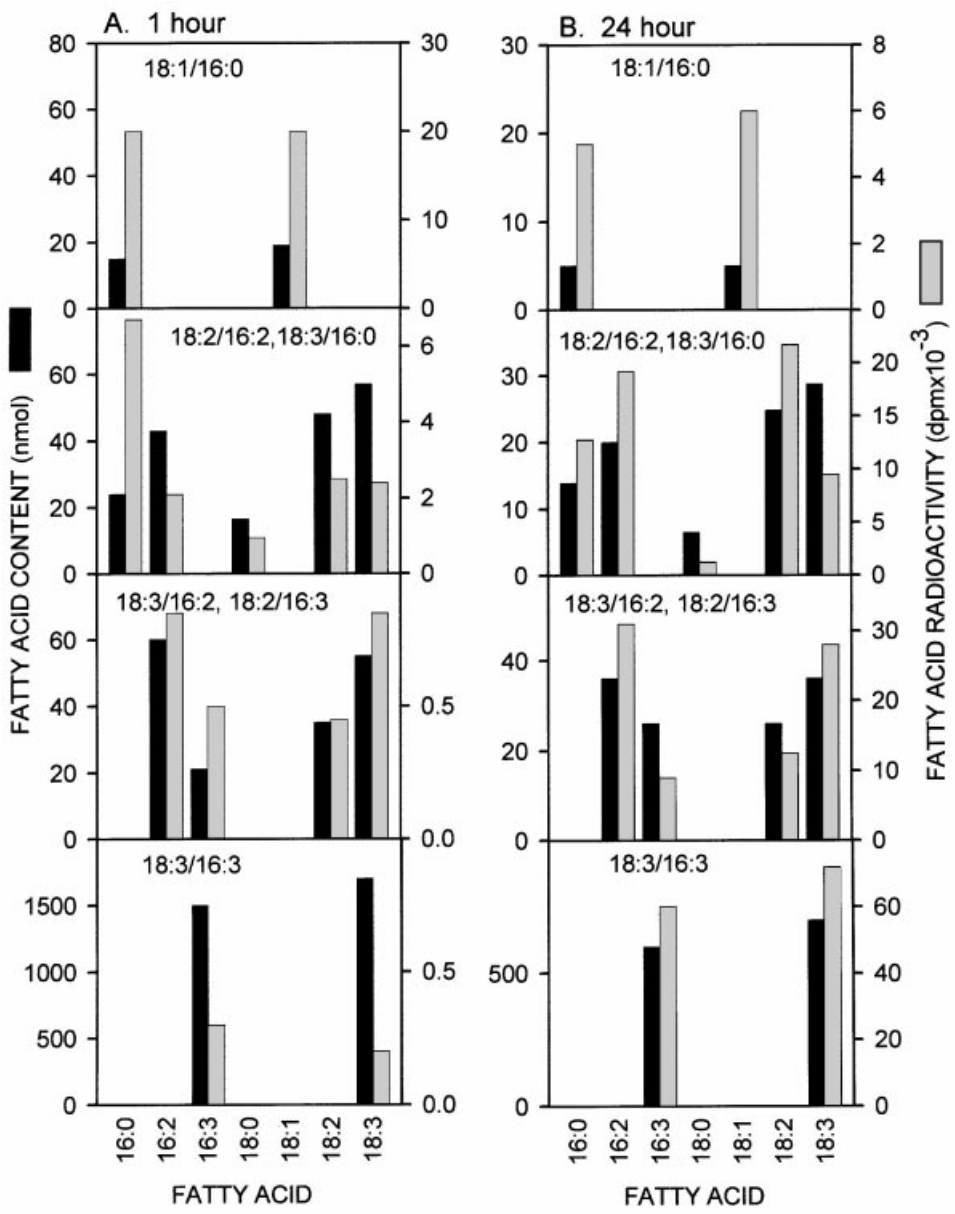


Figure 2 Fatty acid content and radioactivity of fatty acids from argentation TLC bands of prokaryotic molecular species of MGDG after ^{14}C labelling and incubation for (A) 1 and (B) 24 h

Only the major molecular species and fatty acids are presented in each band. Data are from one experiment that is typical of at least three experiments conducted at the same incubation times.

ticularly 18:2/18:3, 16:0/18:2), the higher levels of radioactivity in 18:2 also result in uneven labelling of the two fatty acids. Molecular species containing significant levels of radioactivity in 18:3 follow the same pattern of different levels of radioactivity in the two fatty acids. These data suggest that the fatty acids are continuously exchanged between all molecular species of PC and the acyl-CoA pool immediately after feeding and during longer periods of incubation. We believe this indicates that fatty acids in PC and the acyl-CoA precursor pools are in continuous flux.

Specific radioactivity of fatty acids in PC

The high levels of radioactive 18:1 found in PC immediately following feeding in association with more unsaturated fatty acid can only be interpreted as due to the exchange of newly formed fatty acid in the acyl-CoA pool with existing fatty acid in PC and not solely due to synthesis of new PC. If the fatty acids are exchanged continuously between PC and the acyl-CoA pool it would be expected that each fatty acid, irrespective of the molecular species to which it is esterified, would have the same or similar specific radioactivity. Calculations of specific radioactivity of these fatty acids from each molecular species (Table 2) indicate that the specific radioactivity of each fatty acid, while differing from other fatty acids, is similar regardless of the molecular species in which it is located. In contrast, in MGDG each fatty acid has a different specific radioactivity depending on the molecular species with which it is associated ([16], and results not shown).

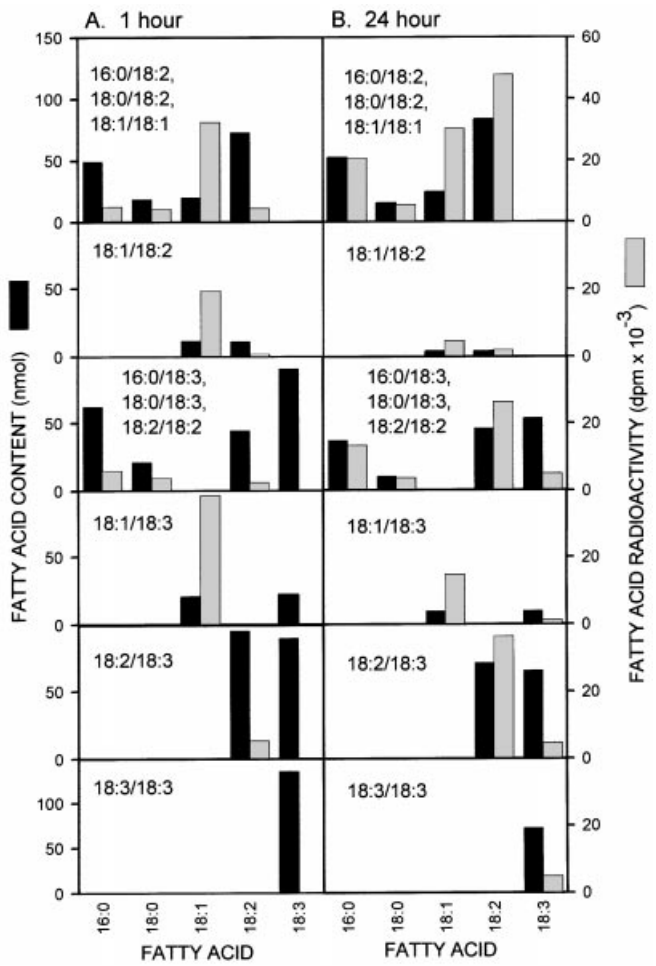


Figure 3 Fatty acid content and radioactivity of fatty acids from the major molecular species and fatty acids from argentation TLC bands of PC after ^{14}C labelling and incubation for (A) 1 and (B) 24 h

Data were obtained from the same experiments as in Figure 1 and are typical of at least three separate similar experiments.

Table 2 Specific radioactivity of fatty acids from the major molecular species of PC from *B. napus* leaves after ^{14}C labelling and incubation for 0, 1 and 24 h

Molecular species were separated as bands by argentation TLC and the data for the major fatty acids in each band are presented. The data are from one experiment that is typical of at least three experiments at each time.

		Specific radioactivity of fatty acids (d.p.m./nmol)					
Incubation time	Band	16:0	18:0	18:1	18:2	18:3	Major molecular species
0 h	1					0.4	18:3/18:3
	2				3	1	18:2/18:3
	3			273		2	18:1/18:3
	4	39	106		10	1	16:0/18:3, 18:0/18:3, 18:2/18:2
	5			248	8		18:1/18:2
	6	33	105	207	11		16:0/18:2, 18:0/18:2, 18:1/18:1
1 h	1					1	18:3/18:3
	2				56	2	18:2/18:3
	3			1893		5	18:1/18:3
	4	96	178		55	2	16:0/18:3, 18:0/18:3, 18:2/18:2
	5			1720	62		18:1/18:2
	6	100	230	1618	62		16:0/18:2, 18:0/18:2, 18:1/18:1
24 h	1					69	18:3/18:3
	2				516	71	18:2/18:3
	3			1510		126	18:1/18:3
	4	362	357		575	94	16:0/18:3, 18:0/18:3, 18:2/18:2
	5			1253	576		18:1/18:2
	6	413	289	1137	514		16:0/18:2, 18:0/18:2, 18:1/18:1

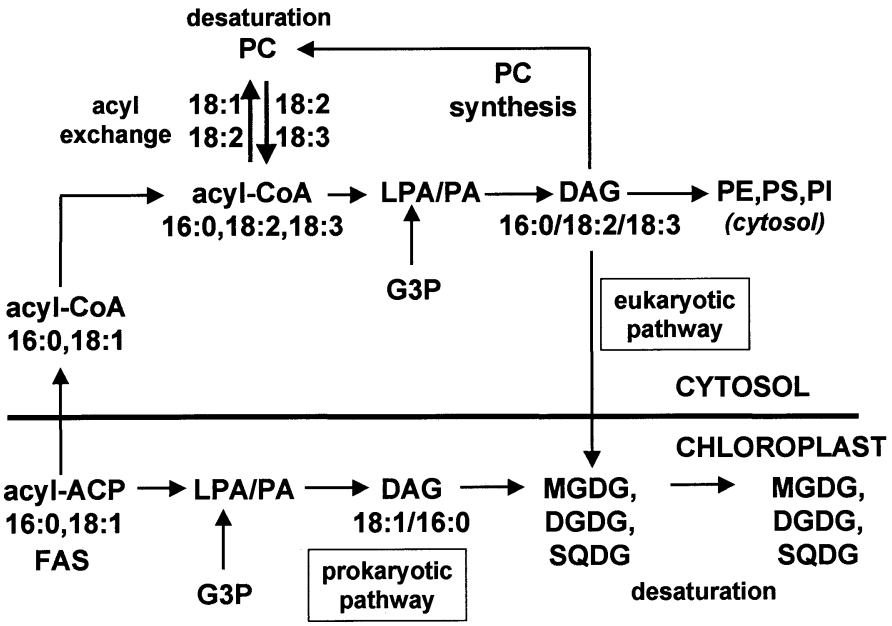
Clearly the specific radioactivity of each fatty acid in PC varies with time. The specific radioactivity in 18:1 decreases with time while the specific radioactivities of 18:2 and 18:3 gradually increase. The fact that after 24 and 48 h of incubation the specific radioactivity of each fatty acid remains similar in different molecular species while changing with time is indicative of continued exchange between PC and the acyl-CoA pool.

DISCUSSION

In many ^{14}C-labelling experiments *in vivo* it has generally been assumed that ^{14}C incorporated into the fatty acids of PC and other lipids from $^{14}CO_2$ or [^{14}C]acetate occurs solely as the result of new synthesis of these lipids [1]. It has also been assumed that the first products of PC synthesis are molecular species containing predominantly 16:0 and 18:1, and that 18:1 is subsequently desaturated to form molecular species containing high levels of 18:2 and 18:3.

From the data presented here on *B. napus* leaves we can make two clear observations that are not consistent with the above assumptions. First, after $^{14}CO_2$ feeding the radioactivity of the two fatty acids on most molecular species of PC have different levels of radioactivity. This can only be the result of the mixing in PC of newly synthesized (radioactive) and existing (non-radioactive) fatty acids by deacylation/reacylation of the lipid molecule. This must be a continuous process as this random mixing is also shown in the higher unsaturated species at later incubation times. Secondly, the specific radioactivities of each fatty acid in different molecular species of PC are similar. This would suggest that all, or a very significant part, of the PC in the cell is involved in this process and that PC may be acting as a large pool of substrate for desaturation and a source of unsaturated fatty acid for the acyl-CoA pool. Although PC is found in most cell membranes outside the chloroplast, it is generally believed that biosynthesis and desaturation occur in the endoplasmic reticulum. However, our data suggest that the cytosolic acyl-CoA pool may exchange fatty acids with PC in many of these membranes.

Our hypothesis and the two different strategies of desaturation in the non-chloroplastic PC and chloroplastic MGDG of *B. napus* are outlined in Scheme 1. In the chloroplast, fatty acids are desaturated in a pool of MGDG predominantly in the envelope. MGDG is synthesized in the prokaryotic pathway from a small metabolic pool of MGDG molecular species containing 18:1/16:0 that represents less than 1% of the total MGDG (Figure 2A and Table 1). The small size of this pool results in the rapid desaturation and the subsequent transfer of the highly unsaturated molecular species to the thylakoid membranes forming the bulk MGDG pool. In our hypothesis the entire pool of PC,



Scheme 1 Outline of the pathways of biosynthesis and desaturation of fatty acids in PC and the chloroplast lipids in *B. napus*, a 16:3 plant

The scheme illustrates the different strategies used in the non-chloroplastic compartments and chloroplast to raise and control the level of unsaturation in lipids. In 18:3 and 18:4 plants the prokaryotic pathway does not participate in the synthesis of MGDG, DGDG and SQDG. The major fatty acid components are indicated in each pool. LPA/PA, lyso-phosphatidic acid/phosphatidic acid; G3P, glycerol 3-phosphate; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; FAS, fatty acid synthase; ACP, acyl carrier protein.

not just a small metabolic pool, is involved in the desaturation process. Through acyl exchange between PC and the acyl-CoA pool, the DAG subsequently formed from the acyl-CoA pool is maintained in a highly unsaturated state. From this pool of DAG, the other glycerolipids in the cell may be synthesized. The levels of unsaturation and rates of desaturation of fatty acid would not be tied to the rates of synthesis and degradation of PC, nor would there be a need for a separate pool of PC undergoing desaturation.

Previous reports using studies *in vitro* of developing soya bean cotyledons [17], safflower seeds [18,19], and sunflower seeds [7] have shown some exchange of fatty acids on the *sn*-2 position of PC with the acyl-CoA pool. In leaves, we believe this is a more extensive process than proposed for seeds, involving nearly all of the PC in the cell and resulting in the synthesis of highly unsaturated DAG. This DAG may be similar in fatty acid composition to the PC in the cell rather than containing just saturated (16:0) and monounsaturated (18:1) fatty acid. We have recently obtained support for the idea that the DAG precursor of PC is highly unsaturated from experiments using ^{32}P labelling in *B. napus* (16:3 plant) leaves [20], and *Vicia faba* (18:3 plant) and *Borago officinalis* (18:4 plant) leaves (J. P. Williams, M. U. Khan, J. N. Hodson and K. M. Hong, unpublished work). These data all show that PC is synthesized from highly unsaturated molecular species of DAG similar to the fatty acid composition of the bulk PC in all three types of plant.

In our experiments the apparent rate of desaturation of 18:2 to 18:3 measured by the rate of transfer of ^{14}C is slow (Table 1) and is not compatible with the rapid rates of incorporation of ^{14}C into PC. The slow incorporation of ^{14}C into 18:3 may be an indication that most of the PC, which is usually high in 18:2, is the substrate for desaturation. Hence the slow rate of incorporation of radioactivity into 18:3 may be due mainly to the large size and low specific radioactivity of the PC 18:2 pool and not due to slow desaturation.

Our hypothesis raises the question of whether the PC is the direct precursor of chloroplast lipids. A pathway from PC to chloroplast lipids, or TAG in seeds, involving a reversal of the CDP-choline:DAG-phosphotransferase has been used to explain how unsaturated fatty acids could be transferred from PC [1,17] in the event that newly synthesized DAG contains saturated and monounsaturated fatty acid. However, if the DAG in the non-chloroplastic compartments is largely composed of unsaturated fatty acid, as we have shown, it is unnecessary for PC to be an intermediate. The chloroplast lipids could be synthesized directly from the DAG pool that contains levels of unsaturated fatty acid generated by, and similar to those in, PC.

Our hypothesis could also explain the slow transfer of ^{14}C fatty acid from PC to MGDG even in developing leaf cells [2,3]. The exchange of radioactivity between PC and other glycerolipids through DAG is dependent not only on the rate of transfer of fatty acid but also the specific radioactivity and the pool size of the PC from which it originates. If the entire PC in the cell is involved in acyl exchange with the acyl-CoA pool, it would represent a large pool of lipid with a relatively low specific radioactivity. Consequently any fatty acid that is transferred from PC would represent only a small part of the total pool and would be low in total radioactivity. This would result in the slow transfer of radioactivity to the acyl-CoA pool and to DAG despite a fairly rapid transfer of fatty acid.

Recently Mongrand et al. [9] have also challenged the generally accepted hypothesis, suggesting that lysophosphatidylcholine (lyso-PC) instead of PC may be transferred to the chloroplast for MGDG and DGDG synthesis. Their hypothesis is partially based on the differential labelling of the fatty acids on the two *sn* positions of PC after ^{14}C-labelling [9] and the demonstration of

an active acyl-CoA:lyso-PC acyl transferase [21] in leaves of leek seedlings. Stymne et al. [4] and Stobart et al. [6] also showed differential turnover or exchange of fatty acid from acyl-CoA with the *sn*-2 position of PC in microsomes from developing safflower seeds. Analyses of molecular species of PC from potato microsomes [19] and sunflower seeds [7] showed similar uneven incorporation of label *in vitro* after feeding with [^{14}C]oleoyl-CoA. Roughan et al. [22], however, showed more even labelling of the two *sn* positions of PC in spinach leaves and similar rates of desaturation on both *sn* positions after incorporation of exogenous [^{14}C]oleic acid. We have shown that in *B. napus* leaves fed $^{14}CO_2$ that the fatty acids on both *sn* positions of PC are labelled and undergo approximately equal apparent rates of desaturation (J. P. Williams and D. Wong, unpublished work). It is possible in our model that the exchange of fatty acid between PC and the acyl-CoA pool occurs unevenly on the two *sn* positions of PC. The presence of significant levels of 16:0 esterified to the *sn*-1 position must also be taken into account, as this would reduce the apparent level of desaturation on the *sn*-1 position. The relative labelling of fatty acids on the two positions would be determined by both the desaturation rates and the deacylation/reacylation rates for each *sn* position. These rates may vary between plant species, different organs and under different physiological and environmental conditions. Active lyso-PC:acyl transferase as reported by Bessoule et al. [21] and Triki et al. [7] would be an important feature of our hypothesis.

Concerning the glycerol/fatty acid labelling, Mongrand et al. [9], Slack et al. [23] and Williams et al. [3] have shown that a decrease in the labelling of glycerol and fatty acid in PC is accompanied by an increase in labelling of these moieties in MGDG. However, accurate relative labelling patterns of fatty acids and glycerol are difficult to determine and may be interpreted in different ways (see [9]). In fact, a re-examination of our data [3] in light of the recent findings suggest that the similar increase in ^{14}C labelling of glycerol and fatty acid in MGDG occurs only at early incubation times. At longer incubation times the increase in fatty acid labelling is not matched by an increase in glycerol labelling. A free exchange of fatty acid in PC with the acyl-CoA pool would mean that the glycerol and fatty acid are not continuously esterified in PC. If the glycerol and fatty acid are transferred to the chloroplast as DAG, the relative labelling of the two moieties would be a reflection of the different kinetics of glycerol and fatty acid metabolism in PC and DAG.

Our hypothesis is that at all times and under changing environmental conditions there would be a constant supply of unsaturated DAG species for lipid biosynthesis. Changes in environmental conditions would influence the fatty acid composition of these pools but would not suddenly increase or decrease the supply of unsaturated DAG. The control over the levels of unsaturated fatty acids may, therefore, be buffered from major changes in temperature and other environmental factors. In MGDG a buffering effect is also present, as the bulk of the lipid in the thylakoid is not immediately affected by environmental change. In both of these systems rapid changes in rates of desaturation are slowly reflected in changes in fatty acid composition of the lipids, thus mitigating rapid changes in unsaturation due to environmental factors.

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