Cellular and thylakoid-membrane phospholipids of *Chlamydomonas reinhardtii* 137+

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Abstract The phospholipids of phototrophically-cultured *Chlamydomonas reinhardtii* 137+ have been quantitated at the cellular and thylakoid membrane levels. The alga contains three major phospholipids, phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), which together constitute about 14% of the cellular polar lipid complement. PG is the only phospholipid in an analytical fraction of thylakoid membrane isolated from the alga, and represents about 9% of the membrane's total polar lipid. Slightly more than half the cellular PG is localized in the photosynthetic lamellae. Hexadecanoic and octadecanoic fatty acids make up over 70% of the acyl groups esterified to these phospholipids, PG being the most highly unsaturated of the three. Differences in fatty acid profile between cellular and thylakoid PG, as well as the presence of PE and PC in the alga but not in its thylakoid, are indicative of a strict biogenetic regulation of the specific types and species of phospholipid associated with the photosynthetic membrane.—**Janero, D. R., and R. Barrnett.** Cellular and thylakoid-membrane phospholipids of *Chlamydomonas reinhardtii* 137+. *J. Lipid Res.* 1981. **22:** 1126–1130.

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Glycoglycerolipids appear to be the major polar acyl lipid type in the relatively limited number of green plant tissues whose lipid biochemistry has been studied. However, the amounts of phospholipid encountered with the glycolipid are not negligible (1). Whereas the glycolipid complement is largely restricted to three individual lipids (MGDG, DGDG, and SL) found almost exclusively in plants, the phospholipids of green plants are more varied and include those which routinely occur in the animal kingdom as constituents of membranes, blood lipoproteins, etc. (2).

Subcellular studies on a few green plant tissues (2, 3) give the general impression that non-green membranes contain significantly higher concentrations of a wider variety of phospholipids than do photosynthetic membranes (thylakoid). The only significant non-glycoglycerolipid in thylakoid is usually PG, and it is open to question whether the small amounts of other phospholipids found in some thylakoid fractions are actually in the green membrane or in contaminating structures (4). Although PG is also found in extra-thylakoid plant membrane, its esterified fatty acids vary with the cellular location of the lipid, and one fatty acid, *trans*-16:1, appears to be concentrated in thylakoid-associated PG (2, 4).

We have presented in a companion report (5) the glycolipid biochemistry of the green algal phytoflagellate *Chlamydomonas reinhardtii* 137+ at the cellular and thylakoid levels. Our results show that in *Chlamydomonas*, as in most green plants studied so far, glycolipid accounts for 70–80% of the total tissue and total thylakoid polar lipid. In higher green plants the remaining polar glycerolipid is phospholipid (2, 6), but in *Chlamydomonas* 137+ the remainder is comprised of the ether lipid, diacylglyceryl-trimethylhomoserine (DGTS)[2] and phospholipid in roughly equal amounts. In this study we show the individual algal and thylakoid phospholipids of phototrophic, wild-type *Chlamydomonas* and assess the intracellular distribution and fatty acid profiles of each.

EXPERIMENTAL PROCEDURES

Phototrophic culture of *Chlamydomonas reinhardtii* 137+, fractionation of the alga, lipid purification, TLC, and quantitation, statistical evaluations, and miscellaneous procedures were as described (5).

Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SL, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; DGTS, diacylglyceryl-trimethylhomoserine; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

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TABLE 1. Quantitation of phospholipids in *C. reinhardtii* 137+

Lipid	μg/mg Chlorophyll		% of Total Polar Lipid		Mass Ratio TM/WC
	WC	TM	WC	TM	
PG	209 ± 14	119 ± 4	7.3 ± 0.5	8.8 ± 0.3	0.57 ± 0.03
PE	143 ± 10	n.d.[a]	5.0 ± 0.3		
PC	39 ± 4	n.d.[a]	1.4 ± 0.1		
Glycolipid	194 ± 110	1029 ± 38	67.8 ± 3.9	79.2 ± 2.9	
Ether lipid	344 ± 25	131 ± 6	13.0 ± 0.8	9.7 ± 0.4	

[a] n.d., none detected.

The mass of each cellular (WC) and thylakoid-membrane (TM) phospholipid as standardized to chlorophyll mass and the ratio for each phospholipid of its thylakoid and cellular masses, so standardized, are tabulated. The percent each phospholipid contributes to the total polar lipid complement of the cell and the thylakoid membrane is computed. For completeness, mass and percent-contribution values of the total glycolipid and total ether lipid at the cellular and thylakoid levels are also included and represent sums of the various individual lipids of each type resolved by TLC (7). All values are means ± S.D.; n ≥ 6.

Identification of the phospholipids

Of the polar lipids resolved in two-dimensional analytical TLC (7) out of a total-algal or total-thylakoid lipid extract, only three cellular lipids and one thylakoid lipid gave a positive reaction to a chromatographic stain for phosphorus (8), had chemically assayable phosphate (9), and were labeled in vivo from $^{32}PO_4^{3-}$ supplied to actively-growing, log-phase algae. Within statistical deviation (~2–3%), all of the $^{32}PO_4^{3-}$ assimilated into total-cellular and total-thylakoid lipid, and all of the chemically quantitated phosphate in the algal and thylakoid lipid extracts, could be accounted for, respectively, by the three cellular and the one thylakoid phospholipid. No additional phosphorus-containing lipids were detectable on analytical TLC at chromatogram loadings whereby a component representing 0.5% of the total lipid mixture could easily be visualized by charring (10) or phosphorus detection spray (8). Therefore, within our limits of analysis, three significant phospholipids were found in the cell, only one of which was associated with the thylakoid. Each phospholipid had an ester group: phosphate ratio of 2:1, as determined chemically (9, 11).

Of the three phosphorus-containing lipids in the cell, only one gave a positive reaction to detection spray for choline (12), contained chemically assayable choline (13), comigrated in two-dimensional TLC (7) with a PC standard, and produced a water-soluble deacylation product which comigrated on paper (14) with a deacylated PC standard (i.e., glycerylphosphorylcholine); this lipid was taken as PC. Only one of the three phospholipids gave a positive reaction to ninhydrin (15), comigrated in TLC (7) with PE standard, and produced a water-soluble deacylation product which comigrated on paper (14) with a deacylated PE standard (i.e., glycerylphosphorylethanolamine); this lipid was taken as PE. The third algal phospholipid, and the only one in the thylakoid, gave a positive periodate-Schiff reaction (16), comigrated in TLC (7) with a PG standard, and yielded upon deacylation a water-soluble product which comigrated on paper (14) with a deacylated PG standard (i.e., glycerylphosphorylglycerol); this lipid was taken as PG. The R_f values in two dimensional TLC for all three phospholipids were in agreement with published migration patterns of the lipids in the same solvent systems (7).

Fatty acid analysis

Fatty acids were analyzed as their methyl ester derivatives prepared by transesterification with 0.5N sodium methoxide (17). The methyl esters were fractionated into subclasses based on unsaturation by argentation TLC (18) and were separated by GLC and identified as described (5).

RESULTS

The contributions that phospholipid makes to wild-type *Chlamydomonas* cellular and thylakoid polar glycerolipid are shown **Table 1**. Three phospholipids, PG, PE, and PC, comprise about 7%, 5%, and 1.5%, respectively, of the total polar lipids of the alga and about 53%, 37%, and 10%, respectively, of the alga's phospholipid complement. The predominant cellular phosphoglyceride, PG, is the sole phospholipid in the thylakoid, about 9% of the total polar glycerolipid of the membrane. In the alga as a whole, phospholipid was at slightly higher concentration than ether lipid, (i.e., DGTS), but in the thylakoid the concentration of ether lipid exceeded that of phospholipid (i.e., PG). As shown in the companion report (5), glycolipid is the main cellular and thylakoid-membrane

TABLE 2. Major fatty acids of *C. reinhardtii* 137+ phospholipids

	Mole Percent of Total Fatty Acids			
	PG		PE	PC
Fatty Acid	WC	TM	WC	WC
14:0	1.7 ± 0.2	1.3 ± 0.2[a]	<1.0	1.7 ± 0.2
14:1	2.0 ± 0.2	1.6 ± 0.1	<1.0	2.1 ± 0.2
14:2	1.6 ± 0.1	1.0 ± 0.1	<1.0	<1.0
14:3	2.1 ± 0.1	<1.0	<1.0	<1.0
16:0	18.5 ± 1.0	13.9 ± 0.7	7.2 ± 0.3	29.2 ± 1.6
16:1	14.5 ± 0.9[b]	20.5 ± 0.9[c]	2.1 ± 0.1	4.1 ± 0.2
16:2	6.8 ± 0.3	3.8 ± 0.2	1.8 ± 0.2	3.3 ± 0.1
16:3	2.4 ± 0.1	2.3 ± 0.2[a]	2.9 ± 0.1	5.6 ± 0.1
16:4	<1.0	2.7 ± 0.1	2.7 ± 0.2	<1.0
18:0	5.6 ± 0.4	4.8 ± 0.1	28.3 ± 1.0	3.6 ± 0.3
18:1	8.1 ± 0.2	6.2 ± 0.2	7.9 ± 0.2	28.0 ± 1.0
18:2	13.6 ± 0.5	18.2 ± 0.6	4.1 ± 0.3	3.6 ± 0.2
18:3	5.6 ± 0.2	13.6 ± 0.4	2.1 ± 0.1	3.0 ± 0.3
18:4	2.2 ± 0.1	2.0 ± 0.1[a]	27.2 ± 0.9	2.5 ± 0.2
20:0	<1.0	<1.0	3.6 ± 0.4	<1.0
20:1	7.7 ± 0.5	1.0 ± 0.1	2.8 ± 0.1	1.7 ± 0.1
20:2	1.9 ± 0.1	1.3 ± 0.1	2.2 ± 0.2	4.0 ± 0.2
20:4	2.3 ± 0.1	2.6 ± 0.3[a]	2.1 ± 0.3	<1.0
22:1	1.1 ± 0.1	1.6 ± 0.1	<1.0	<1.0
22:2	1.3 ± 0.3	<1.0	<1.0	<1.0
22:4	1.1 ± 0.2	1.4 ± 0.2[a]	<1.0	<1.0

[a] Means not significantly different ($P \geq 0.05$; Student's *t*-test) between WC and TM values.

[b] 4.9 ± 0.3 mole percent is the *trans* isomer.

[c] 16.1 ± 0.9 mole percent is the *trans* isomer.

Fatty acids comprising >1.0 mole percent of each cellular (WC) and thylakoid-membrane (TM) phospholipid are quantitated. For each lipid, the sum of the tabulated mole percents is >92%. All values are the mean mole percent ± S.D. for eight determinations. For PG, the difference between the respective cellular and thylakoid-membrane means of any fatty acid is significantly different ($P < 0.05$; Student's *t*-test) unless indicated.

glycerolipid type; the glycolipid:phospholipid ratio is about 5 for the cell and about 9 for the thylakoid.

The intracellular distribution of PG was assessed from the ratio of the mass of PG in the thylakoid to the mass of PG in the cell, both standardized to chlorophyll mass. On the assumption that chlorophyll is localized exclusively in the photosynthetic lamellae, this ratio, 0.57 ± 0.03 (S.D.; n = 6), indicates that the thylakoid contains over half the alga's major phospholipid, PG. However, since the thylakoid contains neither PE nor PC, the ratio between thylakoid PG mass (standardized to chlorophyll mass) and the sum of the masses of all three cellular phospholipids (standardized), 0.3 ± 0.02 (S.D.; n = 6), shows that most (~70%) of the alga's phospholipid is outside the photosynthetic lamellae.

The nature of the fatty acyl groups esterified to phototrophic, wild-type *Chlamydomonas* phospholipids has been investigated by argentation TLC and GLC. As summarized in **Table 2,** all cellular phospholipids and thylakoid PG contain a range of acyl chain lengths from C-14[3] to C-24, with the C-16 and C-18 families accounting for most (>75%) of the phospholipid fatty acids. Thylakoid PG and (cellular) PC have strikingly similar chain length profiles; their C-16 and C-18 acids have a 1:1 ratio. The PG associated with the thylakoid is notably richer in C-18 acids compared to total-algal PG; however, the latter has the greater amounts of C-14 and C-20 acids.

PG is the most highly unsaturated glycerophospholipid, and the PG associated with the thylakoid is even more unsaturated than algal PG as a whole. Since cellular and thylakoid PG contain equal proportions of monoenes and dienes, the greater unsaturation of thylakoid PG is due to its relative enrichment in trienes and tetraenes combined with less unsaturated acids. PE and PC both contain 35–40% saturated acids, giving them the lowest (in fact, identical) unsaturated: saturated ratios, ~1.6. This is to be compared to a value of 25% saturated acids in PG.

In cellular and thylakoid PG, 16:0, 16:1, and 18:2 constitute about 50% of the esterified fatty acyl groups. A greater proportion of 18:3, along with lower amounts of all major saturates, makes thylakoid PG more unsaturated than cellular PG. Of the almost twenty major fatty acids which cellular and thylakoid PG share, only five (14:0, 16:3, 18:4, 20:4, and 22:4) are present in both in equal amounts. PG is the sole phospholipid containing a *trans* unsaturate, *trans*-16:1; about 34% of the 16:1 in cellular PG, but almost 80% of the 16:1 in PG associated with the thylakoid, is the *trans* isomer.

18:0 and 18:4 each comprise ~30% of the major fatty acids in PE and 16:0 and 18:1 comprise ~30% of PC fatty acids. Since one is the major saturated acyl group and the other the major unsaturated acyl group in each lipid, the equal contributions largely account for the equivalence in unsaturated: saturated ratio between PE and PC. This equivalence, however, arises from differing fatty acid complements, since in no case do PE and PC have the same amounts of any major fatty acid which they share.

DISCUSSION

Phospholipid constitutes a fairly small proportion of the polar glycerolipids of phototrophic, wild-type

[3] Two shorthand notations for fatty acids are used. The number of carbon atoms in a fatty acid family is designated numerically (e.g., C-16 denotes the hexadecanoic series). Individual fatty acids are denoted by two numbers separated by a colon. The first number signifies the carbon chain length; the second, the number of unsaturated bonds.

Chlamydomonas reinhardtii, whether in the cell as a whole or in its thylakoid membrane. We can account for all the algal phosphoglyceride by three lipids: PG, PE, and PC. Yet statistical error (ca. 3%) in the associated analytical procedures and limits of reliable chromatographic and chemical detection of a component representing about 0.5% of the total lipid mixture analyzed do not allow us to state unequivocally that other minor phospholipids are not present in the alga. Any such phospholipids, however, would be present at trace levels relative to PG, PE, and PC.

Similarly, the phosphoglyceride associated with the thylakoid fraction can be accounted for solely by PG. We have determined (5)[2] that the green membranes in this fraction are virtually homogeneous, contaminated only by about 3–4% of the total cellular mitochondrial population. The lipid which this contamination contributes to the thylakoid-associated lipid in the fraction must be negligible (i.e., <0.5% of the total fraction lipid), since we can detect in the cell, but not in the fraction, two typical (19) plant mitochondrial phospholipids, PE and PC. The level of sensitivity of our methods and the quality of the thylakoid fraction, therefore, seriously undermine the possibility that phospholipid associated with the thylakoid in addition to PG has gone undetected. Rather, it appears that phospholipids other than PG (mainly PE and PC) ascribed to thylakoid membrane (cf. 4, 6) may be signals of significant thylakoid contamination by non-green membrane. Phospholipid (especially PE, PC, and PI) is found in high concentrations in the chloroplast envelope (3) and in the mitochondrion (19, 20). These structures would be likely sources of such thylakoid contamination as well as likely locations of the PE and PC we have found in *Chlamydomonas* cells, but not in their photosynthetic lamellae.

Phospholipid has been detected in the non-etiolated (and etiolated) *Chlamydomonas* mutant y-1 (21), and provisional chromatographic methods have suggested (22) identification of PG, PE, PC, and PI and PS in this strain. Further, some 75% of total algal phospholipid has been found in a thylakoid fraction prepared from the y-1 mutant (21). Since the individual phospholipid components were not quantitated in any of these studies, it is not known how the phospholipids of wild-type *Chlamydomonas* compare with those of the y-1 strain, a spontaneous mutant of the wild-type (strain 137^+) which we have analyzed.

The only significant cellular phospholipids of the arginine-requiring *Chlamydomonas* mutant ss and the arginine-requiring, streptomycin-resistant strains sr_3 and sr_{35} are PG, PE, and PC (23), as we have found for the wild-type alga. In the three heterotrophic mutants and in the phototrophic wild-type alga, glycerophospholipid comprises about 14% of the total polar lipid and is present in slightly higher quantity than ether lipid, but in far lower amounts than glycolipid. Although between the sr mutants in general and the wild-type alga some similarity of acyl groups exists, each cellular phospholipid has a quantitatively distinct acyl profile. It must yet be determined to what extent such differences among algal strains depend upon physiological (lipid synthesizing capabilities, acyltransferase activities) and/or metabolic (such as the external carbon source present in the mutants' mixotrophic cultures) factors.

Subcellularly, PG and PE have been detected in crude photosystem fractions prepared from *Chlamydomonas* strain sr_3 (24), and phospholipid, at least some of which is PG, has been found in a green membrane pellet from strain 137^+ (25) The quality of the fractions utilized in these studies and the fragmentary nature of the lipid quantitation and identification make comparison between our data at the thylakoid membrane level and these findings difficult.

The quantitative importance of phospholipid in *Chlamydomonas* 137^+, as in other green plants (26–28), is relatively slight compared to glycolipid. Within this generalization, however, the fatty acid profiles of any particular phospholipid may display radical interspecies variations. Perhaps the most striking trends in green-plant phospholipid biochemistry are the related tendencies for PG to concentrate in the thylakoid and for 3-*trans*-16:1 to associate exclusively with the C-2 position of thylakoid PG in actively photosynthesizing tissue (2, 4). Our finding of PG as the sole phospholipid in the thylakoid of wild-type *Chlamydomonas*, with 16:1 its major fatty acid (80% of which is the *trans* isomer), is consistent with these trends, although stereospecific analyses are required to define the position of the *trans* acid in *Chlamydomonas* PG. Also, the data we present do not offer any direct evidence on the speculation (29) that *trans*-16:1 plays a role in thylakoid stacking to form grana.

The differences we and others (4, 20, 30) have observed in the quantities and species of PG associated with various intracellular and even intraorganellar plant membranes must not be overlooked when considering the possible interrelations of PG and membrane physiology. The biogenetic mechanisms which establish a molecularly distinctive phospholipid profile in the *Chlamydomonas* thylakoid are especially intriguing, since this is the only polar lipid class in *Chlamydomonas* whose individual members are not all found in the major cellular membrane, the thylakoid (cf. 5). The sole thylakoid phospholipid is the cellular anionic phospholipid, PG; PE and PC are excluded.

Maintenance of thylakoid phospholipid biochemistry in *Chlamydomonas* would involve, therefore, strict coordinate regulation of both the phospholipid species and the individual phospholipid types assembled into the membrane.

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