Positional distribution of fatty acids in the glycerophospholipids of *Tetrahymena pyriformis*

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Abstract The positional distributions of the fatty acids in the major glycerophospholipids of *Tetrahymena pyriformis* W were analyzed. A comparison was made of the acyl distributions in normal and ergosterol-grown cells. It was assumed that the positional arrangement of fatty acids would serve as an indicator of acyltransferase enzyme specificity. The acyltransferases in this protozoan have substrate specificities that direct unsaturated groups, particularly polyunsaturates, to the 2-carbon of the glycerophospholipids. An exception is γ-linolenic acid, which represents a substantial proportion of the total acids at both carbons. Saturated and *iso*-acids are esterified primarily at the 1-carbon. The qualitative pattern of the fatty acyl distribution is the same in both normal and ergosterol-grown organisms. Sterol substitution produces quantitative differences in the acyl components at both the 1- and 2-carbons of the glycerophospholipids. These differences include a shortening of the average chain length and a decrease in total unsaturation at both the 1- and 2-positions. In addition, there is a modification at the 2-carbon in the relative amounts of the products of two pathways involved in the biosynthesis of fatty acids. The data are interpreted to indicate that the fatty acid transformations in the glycerophospholipids of organisms that contain ergosterol are not the result of altered acyltransferase specificities. —**Pieringer, J., and R. L. Conner.** Positional distribution of fatty acids in the glycerophospholipids of *Tetrahymena pyriformis*. *J. Lipid Res.* 1979. **20:** 363–370.

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Experiments with the ciliate, *Tetrahymena pyriformis*, have shown that the addition of a variety of sterols to the growth medium inhibits the biosynthesis of and replaces tetrahymanol and diplopterol, the native membrane pentacyclic triterpenoid alcohols (1–3). Ergosterol, an effective sterol, is accumulated by the cells and appears solely as the free alcohol. No esterification or further metabolism occurs (4, 5). This substitution causes an alteration in the fatty acyl composition of several glycerophospholipids, namely, 1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate, 1-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate, 1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphate and, to a lesser extent, of 1,2-diacyl-*sn*-glycero-3-phosphorylcholine and 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine. Both the average fatty acid chain length and the relative amount of unsaturated acids are decreased in ergosterol-supplemented cells. In addition, a differential effect on unsaturated fatty acids was noted which is related to the two biosynthetic pathways found in this ciliate (5). In one case palmitate is elongated to stearic acid and successive desaturations lead to the formation of oleic ($18:1\Delta^{9}$), linoleic ($18:2\Delta^{9,12}$), and γ-linolenic ($18:3\Delta^{6,9,12}$) acids (6–8). The second pathway involves the direct desaturation of palmitate to palmitoleate ($16:1\Delta^{9}$) which, in turn, may be further desaturated to produce two 16:2 isomers and $16:3\Delta^{6,9,12}$ or elongated to *cis*-vaccenate ($18:1\Delta^{11}$). The latter acid is desaturated to yield cilienic acid ($18:2\Delta^{6,11}$) (8). Cells supplemented with ergosterol show an enrichment in palmitoleic acid and its derivatives and a diminution of stearic acid products (5).

The impact of sterol supplementation on the fatty acyl composition can be explained by one of three processes: *1*) an alteration in the incorporation of fatty acids into phospholipids by a change in acyltransferase specificity; *2*) a change in fatty acid synthesis per se

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; PE, 1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphate and 1-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphate; PnE, 1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate and 1-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate; PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine and 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; BHT, butylated hydroxytoluene; *iso*-fatty acids are indicated by (*i*) after the fatty acid designation, e.g., 17:0(i).

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which may determine the availability of particular fatty acyl groups for phospholipid biosynthesis; and *3*) an effect on the turnover of fatty acyl groups of phospholipids so that certain acids are replaced more rapidly than others. The additional possibility exists that some combination of these three processes takes place.

Knowledge of the positional distribution of the fatty acyl residues in the glycerophospholipids was deemed necessary to examine the first of these possibilities. This would permit a change in the specificity or activity of the acyltransferases in the presence of ergosterol to be detected. Such an analysis would reveal whether ergosterol supplementation leads to the differences in the fatty acid pattern at the 1-carbon, the 2-carbon, or involves both positions of the glycerolipids. A complete report on the fatty acid positional distribution in the polar lipids of the ciliate is not available, although information for phosphatidylcholine in another species has recently appeared (9). Our report lists the fatty acyl positional distribution for all the major glycerolipids isolated from both control and ergosterol-supplemented ciliates. The data suggest that the fatty acyl shifts observed in sterol-supplemented cells are not due to a change in the specificity of the acyltransferases.

MATERIALS AND METHODS

General

All organic solvents and glacial acetic acid were redistilled before use. Solvents used in extraction and fractionation of lipids contained an antioxidant, BHT (Shell Fine Research Chemicals, Houston, TX) at a final concentration of 8 μg/ml (10). BHT was not added in subsequent procedures to avoid its appearance as a contaminant in fatty acid analysis (11).

Ergosterol (Sigma Chemical Co., St. Louis, MO) was recrystallized three times from methanol or acetone (mp 162–164°C) and purity was verified by GLC as previously described (2). Unisil (100–200 mesh, Clarkson Chemical Co., Williamsport, PA) and SilicAR (cc-4, 100–200 mesh, Mallinckrodt Chemical Works, St. Louis, MO) were washed with methanol at 65°C and reactivated overnight at 110°C to remove material that interfered with fatty acid analyses.

Removal of organic solvents was accomplished with a rotary evaporator operated under reduced pressure, or with a stream of nitrogen. Lipid samples were stored in the dark under nitrogen at −20°C.

Growth of *Tetrahymena pyriformis* W

Cultures of *Tetrahymena pyriformis* W were grown in 2800-ml Fernbach flasks which contained 500 ml of an enriched proteose–peptone medium (12). Five mg of ergosterol dissolved in 1 ml of absolute ethanol was added to the medium to obtain supplemented cultures (5). Cells were harvested after a growth period of 22–24 hr (deceleration phase of growth) at 25 ± 1°C. Procedures for estimation of cell numbers, harvesting, lyophilization of cells, and extraction of lipids have been described (2).

Fractionation of lipids

Nonlipid contaminants were removed from the lipid extracts by filtration through a column of Sephadex G-25 (Pharmacia, Piscataway, NJ) (13). The purified extracts were fractionated on columns of Unisil (1.5 g Unisil/100 mg lipid) (5). The sample was applied to the column in chloroform and lipids were eluted with the following solvents in sequence: chloroform, chloroform–acetone 2:1, acetone–methanol 9:1, chloroform–methanol 14:1, chloroform–methanol 8:1, and chloroform–methanol 2:1. All solvent mixtures reported in this paper are on a per volume basis. The composition of each fraction was ascertained by thin-layer chromatography on silica gel G plates 0.25 mm thick (EM Laboratories, Elmsford, NY) developed in chloroform–methanol–water 95:35:4 (14). Lipids were visualized on the plates by staining with 2% ninhydrin in *n*-butanol or Dragendorff reagent (15) for compounds with amine or choline groups, respectively. Other lipids were located by spraying with 15% phosphomolybdate in absolute ethanol, followed by charring at 110°C.

Phosphatidylethanolamine (PE) and its phosphonate analogue (PnE) were found in the chloroform–methanol 14:1 fraction from the Unisil column. This mixture was further resolved on a column of SilicAR cc4 (16). A sample of 200–400 mg was dissolved in chloroform–glacial acetic acid 3:1 and applied to a 40-g column (20 mm i.d. by 65 cm) of SilicAR; the same solvent was used for elution. The eluate was collected in 4-ml portions and progress of the elution was followed by qualitative TLC of aliquots of the fractions on silica gel G plates developed with chloroform–acetic acid–methanol–water 75:25:5:1.5 (17). Lipids were visualized on the plates with ninhydrin. Appropriate fractions were combined to obtain chromatographically pure samples of PnE and of PE.

Ninety-six to one hundred percent of the total lipid phosphorus applied to the SilicAR column was recovered in the eluted material. The purified PnE and PE fractions represented 25% and 60%, respectively, of the recovered lipid phosphorus; 15% of the column eluate included a mixture of both lipids. The phosphonate content of the overlap region ranged from 25 to 30% in the various experiments; therefore, this fraction showed the same proportions of the two phos-

phorus classes as the original material applied to the column.

Phosphatidylcholine was found in the chloroform–methanol (2:1) fraction from the Unisil column, and was purified further by ion-exchange chromatography on TEAE cellulose (Sigma Chemical Co., St. Louis, MO) (18). A suspension of TEAE cellulose in glacial acetic acid was packed into a column, 20 mm i.d., to a height of about 20 cm. A lipid sample of 100–200 mg was dissolved in chloroform and applied to the washed column (18). Elution was carried out with chloroform–methanol 14:1 and chloroform–methanol 9:1. The eluates were collected in 50-ml fractions. The fractions that contained pure PC were identified by TLC of aliquots of each fraction on silica gel G plates developed in chloroform–methanol–water 95:35:4. The plates were sprayed first with Dragendorff reagent, then with ninhydrin, and finally were charred with sulfuric acid to ascertain purity.

In agreement with a previous investigation (5), which included a quantitative two-dimensional TLC analysis of the cellular polar lipids, the PnE, PE, and PC contents were found to be 20%, 40%, and 15%, respectively. Column separation procedures were employed rather than TLC because the recovery of polyunsaturated fatty acids had been found to be consistently higher by the former method (5, 20).

Fatty acid analysis

The procedures for methanolysis of the glycerophosphatides and for separation of the fatty acid methyl esters from residual lipids have been described (19). Free fatty acids were methylated by reaction with diazomethane. Fatty acid methyl esters were analyzed by GLC (20). Each peak in the GLC profiles was cut out and weighed, and the individual fatty acid content of each sample was expressed as percent by weight of the total fatty acids. The significance of the difference between fatty acid compositions of unsupplemented cells and of cells grown with ergosterol was shown by the two-sample t test with the level of significance set at 0.05.

Some of the chromatographic peaks obtained were composites of two methyl esters with similar retention times. The methyl esters of 16:1Δ^9 and 17:0(*iso*) were not resolved under the conditions used in these experiments. The total amount of these two fatty acids was first determined. The relative amount of the 17:0(*iso*) component in each sample was calculated after the mixtures of fatty acid methyl esters were hydrogenated and rechromatographed on GLC. Since unsaturated *iso*-acids correspond to less than 1% of the total, all *iso*-acids reported in this paper were assumed to be saturated (21). The amount of 16:1Δ^9 was obtained by subtraction of the 17:0(*iso*) constituent from the total peak weight. Further, the 18:1 component represented a mixture of two isomers, 18:1Δ^9 and 18:1Δ^{11}, that were not separated (8). The 16:2$\Delta^{9,12}$ constituent included some unresolved 17:1 methyl ester (5).

Phosphorus analysis

The phosphorus content of the lipids was determined by the method of Aalbers and Bieber (22), which incorporated the ashing procedure of Ames (23) to determine total phosphorus, and the sulfuric acid digestion of Bartlett (24) to determine ester phosphorus for any given lipid preparation. The percentage of phosphonate was calculated by subtracting the micromoles of orthophosphate obtained by sulfuric acid digestion (ester phosphorus) from the value obtained by the ashing procedure (total phosphorus) and dividing by the total phosphorus value.

Glyceryl ether analysis

The glyceryl ether content of the phospholipids was measured by a modification of the method of Wood and Snyder (25) as reported by Ferguson et al. (5), with the exception that methyl boronic acid derivatives were prepared for GLC analysis rather than the trimethylsilyl ethers.

Determination of the positional distribution of the fatty acids

The positional arrangements of the fatty acids in PE, PnE, and PC were determined by enzymatic release of the fatty acid esterified to the 2-carbon of the glycerophospholipids according to the method of Uthe and Magee (26). A solution (3 mg/ml) of lyophilized snake venom (*Crotalus adamanteus*, Sigma Chemical Co., St. Louis, MO) was mixed with an equal volume of 10 mM $CaCl_2$. This solution was incubated in a gyrotary shaker at 37°C for 30 min. Five ml of 0.2 M glycylglycine, pH 7.5, and 3 ml of an aqueous solution of sodium deoxycholate (10 mg/ml) were added to 5–10 mg of dried lipid sample (5–15 μmol P). The mixture was sonicated for 1 min with a Branson sonifier (Branson Instruments, Inc., Stamford, CT). Two ml of enzyme solution were added to this emulsion. The mixture was placed in a 25-ml Erlenmeyer flask which was flushed with nitrogen and tightly stoppered. The solution was incubated at 37°C in a gyrotary shaker. Incubation time for PE and PC was 6–8 hr and for PnE, 9–10 hr. The progress of the hydrolysis was followed by TLC of aliquots of the reaction mixture on silica gel G plates, 7.5 cm × 2.5 cm. The plates were developed in chloroform–methanol–water 65:35:5 (27).

After hydrolysis the lipids were extracted into chloroform from the aqueous reaction mixture by the method

TABLE 1. Glyceryl ether content of three lipid classes from *Tetrahymena pyriformis* W grown with and without ergosterol

Phospholipid	Ergosterol +	Ergosterol −
	% Glyceryl ether	
PnE[a]	59.8 ± 8.5[d] (8)[e]	61.7 ± 7.4 (7)
PE[b]	2.3 ± 0.7[d] (6)	3.3 ± 1.8 (6)
PC[c]	60.4 ± 9.3[d] (10)	59.5 ± 9.2 (11)

[a] PnE: Mean ± SD; four cell populations.
[b] PE: Mean ± SD; four cell populations.
[c] PC: Mean ± SD; three cell populations.
[d] There is no significant difference between any of the pairs at $P = 0.05$.
[e] Numbers in parentheses represent the number of determinations from the cell populations studied.

of Bligh and Dyer (28). The products of the reaction were isolated and separated from remaining, unhydrolyzed diacyl phospholipid on a Unisil column. Free fatty acids were eluted with chloroform; unhydrolyzed PE or PnE with chloroform–methanol 14:1; unhydrolyzed PC with chloroform–methanol 4:1; and lysophospholipids with chloroform–methanol 2:1. Purity of each eluate was determined by TLC. Aliquots of each fraction were spotted on silica gel G plates, 7.5 cm × 2.5 cm, which were developed in chloroform–methanol–water 65:35:5 in experiments with PE or PnE, or in chloroform–methanol–acetic acid–water 50:28:10:5 in experiments with PC. Any fraction that contained more than one lipid species was rechromatographed. One half of each lysophospholipid and diacylphospholipid fraction was used for fatty acid analysis, and the other half for phosphorus assay. No more than 3% of the original amount of lipid phosphorus was recovered as unhydrolyzed polar lipid in any experiment.

RESULTS

Isolation of phospholipids

The PE and PnE obtained from SilicAR column chromatography contained no other lipids that could be detected by TLC analysis. Phosphorus assay showed that the PnE contained >97% phosphonolipid and the PE represented >98% phosphorus ester (average of analyses of four preparations of each lipid). The chromatographically pure PC isolated by TEAE cellulose contained <4% phosphonolipid (analyses of three preparations), a finding in agreement with previous investigations (5, 14, 29).

Glyceryl ether analysis

The phospholipids of *Tetrahymena pyriformis* W with 1-alkyl substituents are derivatives solely of chimyl alcohol (14). Of the three classes of phospholipids isolated in this study, only PnE and PC contain substantial amounts of species with an ether linkage at the 1-carbon (**Table 1**). The relative amounts of phospholipid molecules with 1-alkyl groups did not change in response to substitution of ergosterol for tetrahymanol (Table 1) (5).

Fatty acid composition and positional distribution of PE

The fatty acid composition of PE from control cells is shown in the first column of **Table 2.** Approximately 70% were even-numbered, normal, unsaturated acids. The PE from cells grown with ergosterol showed an

TABLE 2. Composition (%) and positional distribution of the fatty acids of 1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphate and 1-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphate

Fatty Acid	Total − [a]	Total +	2-Position −	2-Position +	1-Position −	1-Position +
			Weight %			
12:0	2.2[b]	3.1	5.2	7.7	0.3	0.6
14:0	9.8	12.0	4.2	4.5	14.6	19.0
15:0 (i)	2.2	2.1	0.9	0.9	3.8	4.0
16:0	7.6	7.2	3.2	3.1	15.9	15.4
17:0 (i)	4.4	3.7	2.7	3.2	8.6	5.7
17:0	1.0	1.7	1.0	2.2	1.0	1.5
18:0	1.7	1.9	1.4	1.1	4.0	4.3
16:1Δ^{9}	9.8	10.9	12.3	14.6	4.6	4.7
16:2$\Delta^{6,9}$	0.8	1.4	1.2	2.0	0.2	0.4
16:2$\Delta^{9,12}$	1.1	0.9	1.5	1.5	0.6	0.5
18:1	9.4	10.0	14.9	15.3	3.9	3.1
18:2$\Delta^{6,11}$	3.2	3.7	4.0	4.4	1.6	2.1
18:2$\Delta^{9,12}$	14.9	11.4	24.1	19.0	4.1	3.2
18:3$\Delta^{6,9,12}$	28.1	26.1	20.0	16.9	34.5	32.9
Other[c]	4.1	4.2	3.5	3.6	2.3	2.4
Unsaturated acids	69.9	66.6	80.1	75.7	50.5	47.5
Iso-acids	7.2	6.7	4.5	5.2	12.9	10.4
$<C_{18}$[d]	40.5	45.4	34.2	42.1	50.9	53.9
16:1Δ^{9} pathway[e]	15.1	17.2	19.4	22.9	6.9	7.8
18:0 pathway[f]	43.0	37.5	44.1	36.0	38.6	36.2

[a] Control cultures, −; cultures supplemented with ergosterol, +.
[b] Total: numbers represent the mean of 10 determinations from three cell populations, while the figures for the 2- and 1-position represent the mean of eight determinations from four cell populations. Pairs of numbers (representing control and supplemented cultures) whose difference is significant at $P = 0.05$ or better are underlined.
[c] Includes 13:0(*i*); 13:0; 14:0(*i*); 15:0; 16:0(*i*); 14:1; 16:3$\Delta^{6,9,12}$; and 20-carbon unsaturated fatty acids.
[d] $<C_{18}$: percent acids possessing fewer than 18 carbons.
[e] 16:1 pathway includes 16:1Δ^{9}; 16:2$\Delta^{6,9}$; 16:2$\Delta^{9,12}$; 16:3$\Delta^{6,9,12}$; and 18:2$\Delta^{6,11}$.
[f] 18:0 pathway includes 18:2$\Delta^{9,12}$; and 18:3$\Delta^{6,9,12}$.

increase in the relative amount of fatty acids with fewer than 18 carbons and a decrease in unsaturated fatty acids due to a decline in the amount of linoleate and γ-linolenate (Table 2, column 2). The net result was a reduction in the total constituents of the stearate (18:0) pathway and an augmentation of the components of the palmitoleate (16:1Δ^9) pathway.

Eighty percent of the fatty acids at the 2-carbon of PE were unsaturated (Table 2, column 3). Dienes and monoenes were located preferentially at that position. PE contained a larger amount of monoenes and a smaller proportion of γ-linolenate than the other two phospholipids (column 1 of Tables 2, 3, and 4). Ergosterol supplementation of the cells resulted in a reduction in chain length and unsaturation as well as a rise in components of the palmitoleate pathway concomitant with a diminution in those of the stearate pathway at the 2-carbon of PE (Table 2, column 4).

Approximately one-third of the fatty acid residues at the 1-position of PE was γ-linolenate (Table 2, column 5), similar to the situation in PnE and PC (column 5 of Tables 3 and 4). Equal amounts of saturated and unsaturated acids were esterified to the 1-carbon. The average chain length was shorter at the 1-position than at the 2-position. Saturates and *iso*-acids were preferentially located on the 1-carbon with the exception of 12:0, which was found almost entirely at the 2-carbon. The effect at the 1-position attributable to the substitution of ergosterol for tetrahymanol was a decrease in unsaturation and in chain length (Table 2, column 6). There was no change at this carbon in the proportions of unsaturated fatty acids from the two biosynthetic pathways.

TABLE 3. Composition (%) and positional distribution of the fatty acids of 1,2-diacyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate and 1-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate

	Total		2-Position		1-Position	
Fatty Acid	−	+	−	+	−	+
			Weight %			
12:0	0.2[a]	0.1	0.7	0.5	0	0
14:0	3.0	3.9	0.8	0.8	9.8	13.9
15:0(*i*)	0.8	0.7	0.1	0.1	2.8	2.7
16:0	3.7	3.8	1.3	1.5	17.1	18.2
17:0(*i*)	2.4	2.2	0.8	1.2	7.4	5.5
17:0	0.5	0.9	0.5	0.9	1.1	1.6
18:0	1.0	1.0	0.9	0.9	4.2	4.8
16:1Δ^9	3.3	4.5	3.8	4.8	5.3	5.7
16:2$\Delta^{6,9}$	0.5	1.0	0.6	1.2	0.2	0.4
16:2$\Delta^{9,12}$	0.5	0.6	0.7	0.7	0.5	0.5
18:1	6.6	7.1	8.2	8.9	4.5	3.9
18:2$\Delta^{6,11}$	20.7	25.6	23.6	29.4	4.0	3.5
18:2$\Delta^{9,12}$	14.8	10.9	19.6	14.1	4.5	3.3
18:3$\Delta^{6,9,12}$	39.6	36.0	36.1	33.2	36.8	34.4
Other[b]	2.4	1.6	2.3	1.8	1.7	1.5
Unsaturated acids	88.0	86.9	94.5	93.6	56.4	51.9
Iso-acids	3.2	3.0	1.1	1.6	10.5	8.7
$<C_{18}$[c]	15.6	18.5	10.0	12.5	45.4	49.9
16:1Δ^9 pathway	25.3	31.9	28.9	36.4	10.1	11.1
18:0 pathway	54.4	46.9	55.7	47.3	41.3	37.8

[a] All numbers represent the mean of eight determinations from four cell populations.
[b] Includes 15:0; 16:0(*i*); 14:1; 16:3$\Delta^{6,9,12}$; and 20-carbon unsaturated acids.
[c] See Table 2 for explanation of all other terms and symbols.

Fatty acid composition and positional distribution of PnE

Almost 90% of the total fatty acids of PnE were unsaturated with the 18:2 isomers and γ-linolenate predominating (**Table 3,** column 1). The isomer 18:2$\Delta^{6,11}$ was present in the largest amount in this glycerolipid. Ergosterol supplementation resulted in a reduction in overall fatty acid chain length, an enhancement of the constituents of the palmitoleate pathway, and a decrease in the fatty acids of the stearate pathway (Table 3, column 2). There was no net change in the degree of unsaturation but, rather, a substitution of one series of unsaturated fatty acids for another.

More than 90% of the fatty acids at the 2-position of PnE were unsaturated (Table 3, column 3). Dienes were found primarily at the 2-carbon, but monoenes were more evenly distributed between the 1- and 2-positions. Growth of the organism with ergosterol resulted in an increase in the elements of the palmitoleate pathway with a decrease in the components of the stearate pathway (Table 3, column 4). This shift leads to an increase in fatty acids with fewer than 18 carbons, primarily because of a decline in the quantity of γ-linolenate. There was a replacement of a significant amount of linoleate with the 18:2$\Delta^{6,11}$ isomer at this position.

The polyunsaturate, γ-linolenate, was the most abundant fatty acid at the 1-carbon of PnE, as it was in the other lipids (Table 3, column 5). The saturated fatty acids, 14:0 and 16:0, and the *iso*-acids were esterified primarily at the 1-position. Seventy-eight percent of the total constituents at the 1-carbon, which included hexadecyl ether and acyl residues, were saturated (Table 5). Supplementation of the organism with ergosterol resulted in an enhancement of the quantity of shorter chain, saturated acids at the 1-carbon of PnE (Table 3, column 6). No effect was observed on the contribution of each of the biosynthetic pathways for unsaturated fatty acids.

TABLE 4. Composition (%) of positional distribution of the fatty acids of 1,2-diacyl-*sn*-glycero-3-phosphocholine and 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine

Fatty Acid	Total		2-Position		1-Position	
	−	+	−	+	−	+
	Weight %					
12:0	0.6[a]	0.4	0.9	0.6	0	0
14:0	4.9	4.9	1.4	1.4	14.2	18.7
15:0(*i*)	1.3	1.1	0.4	0.3	4.1	4.0
16:0	4.3	3.8	1.8	1.4	16.0	17.0
17:0(*i*)	3.0	3.0	2.3	3.0	8.7	7.2
17:0	1.0	1.9	1.0	2.0	1.2	1.9
18:0	1.2	1.1	0.8	0.7	4.0	4.2
16:1Δ^{9}	6.9	6.1	5.5	5.3	7.1	5.4
16:2$\Delta^{6,9}$	0.9	1.8	1.0	2.1	0.2	0.2
16:2$\Delta^{9,12}$	0.8	0.5	0.8	0.6	0.6	0.6
18:1	7.5	7.0	8.1	8.7	4.2	3.5
18:2$\Delta^{6,11}$	7.4	7.9	8.9	8.7	2.2	1.5
18:2$\Delta^{9,12}$	12.5	9.5	14.8	11.0	6.8	4.5
18:3$\Delta^{6,9,12}$	44.8	48.5	48.9	50.1	27.7	28.9
Other[b]	2.9	2.3	3.2	3.7	2.9	2.3
Unsaturated acids	83.1	83.0	90.9	90.0	50.5	46.0
Iso-acids	4.6	4.4	3.0	3.6	13.2	11.4
$<C_{18}$[c]	24.9	24.9	16.7	18.4	53.5	56.2
16:1Δ^{9} pathway	16.5	16.9	17.1	17.7	10.2	7.8
18:0 pathway	57.3	58.0	63.7	61.1	34.5	33.5

[a] Total: numbers represent the mean of seven determinations from three cell populations, while the figures for the 2- and 1-position represent the means of five determinations from three cell populations.

[b] Includes 13:0(*i*); 16:0(*i*); 15:0; 16:3$\Delta^{6,9,12}$; and 20-carbon unsaturated fatty acids.

[c] See Table 2 for explanation of all other terms and symbols.

Fatty acid composition and positional distribution of PC

γ-Linolenate constituted almost half of the fatty acids in PC (**Table 4,** column 1). Over 80% of the total fatty acids were unsaturated. No net modification in average chain length, in the degree of unsaturation, or in the constituents of either biosynthetic pathway was effected in the fatty acids of PC by supplementation with ergosterol (Table 4, column 2).

Unsaturated fatty acids represented 90% of the total at the 2-carbon of PC (Table 4, column 3). Alterations in the fatty acid pattern at this position due to ergosterol were minimal (Table 4, column 4).

The fatty acid composition at the 1-carbon of PC resembled that of the other lipids in that the saturates, 14:0 and 16:0, as well as the *iso*-acids were found primarily at that position (Table 4, column 5). The fatty acid present in greatest amount at this carbon was γ-linolenate. Almost 80% of the total constituents at the 1-carbon of PC were saturated hydrocarbon chains when both alkyl and acyl residues were included in the calculations (**Table 5**).

DISCUSSION

The positional distribution of the fatty acyl groups in each of the major glycerophospholipids of *Tetrahymena pyriformis* W is qualitatively similar. Unsaturated fatty acids are the predominant constituents at the 2-carbon of the three lipid classes, and this is especially evident in PnE and PC. Dienes and monoenes are esterified primarily to the 2-carbon in each class of lipid with the exception of palmitoleate, which is evenly distributed between the 1- and 2-carbons of PnE and PC.

The localization of γ-linolenate is striking in that it constitutes a substantial proportion of the acids found at both the 1- and 2-carbons of all three phospholipids. γ-Linolenate is the most abundant fatty acyl residue at the 1-carbon of all three phosphatide classes.

The majority of the total components at the 1-carbon of PnE and PC are hexadecyl ether residues, while PE has only a small amount of this constituent. Saturated fatty acids, both normal and iso, are located primarily at the 1-carbon in each of the phospholipids. The resultant fatty acyl content of PE at this position is approximately a 1:1 ratio of saturated to unsaturated acids, while PnE (78%) and PC (80%) show a higher content of saturated residues.

These data suggest that the acyltransferases involved in the synthesis of the phospholipids in this organism

TABLE 5. Composition of the constituents at the 1-position of PnE and PC from *Tetrahymena pyriformis* W grown with and without ergosterol

Constituent	PnE[b]		PC[b]	
	−	+	−	+
	Weight %			
Hexadecyl ether[a]	61.6	59.8	59.5	60.4
14:0	3.8	5.6	5.8	7.4
15:0(*i*)	1.1	1.1	1.7	1.6
16:0	6.6	7.3	6.5	6.7
17:0(*i*)	2.8	2.2	3.5	2.9
17:0	0.4	0.6	0.5	0.8
18:0	1.6	1.9	1.6	1.7
16:1Δ^{9}	2.0	2.3	2.9	2.1
18:1	1.7	1.6	1.7	1.4
18:2$\Delta^{6,11}$	1.5	1.4	0.9	0.6
18:2$\Delta^{9,12}$	1.7	1.3	2.8	1.8
18:3$\Delta^{6,9,12}$	14.1	13.8	11.2	11.4
Other[c]	0.9	1.0	1.5	1.2
Saturated hydrocarbon chains	78.3	79.1	79.5	81.8

[a] PnE ether: mean of seven determinations from four cell populations. PC ether: mean of 10 determinations from three cell populations.

[b] PnE fatty acids: mean of nine determinations from four cell populations. PC fatty acids: mean of five determinations from three cell populations.

[c] Includes 13:0(*i*); 13:0; 14:0(*i*); 16:0(*i*); 15:0; 16:2 isomers and traces of unsaturated fatty acids with 20 carbons. See Table 2 for explanation of other terms and symbols.

have a substrate specificity that results in the placement of saturates and *iso*-acids at the 1-carbon and in the assignment of unsaturated groups, particularly polyunsaturates, to the 2-carbon, in agreement with the conclusion of Okuyama and co-workers (9) for PC. There is one marked exception to this pattern, namely, the occurrence of large quantities of γ-linolenate at the 1-carbon. Two explanations for this phenomenon can be proposed. First, two enzymes may function, one to attach short chain saturates, and the other to esterify unsaturates, principally γ-linolenate. Alternatively, there may be a single enzyme with a selectivity for saturates which, once in place, may be subjected to the action of one or more desaturases specific for lipid-bound residues. Direct desaturation of esterified fatty acids has been demonstrated in several organisms (30–33). Further work is in progress to determine the correct explanation.

The qualitative patterns of fatty acyl distribution were the same in the phospholipids of both normal and sterol-supplemented cells, but quantitative differences appeared. The influence of sterol supplementation on the fatty acid composition was exerted at both the 1- and 2-positions of PE and PnE. There was a reduction in chain length and an increase in saturated acids at the 1-carbon, due primarily to an increase in the 14:0 component. This effect, an enhancement of a short chain saturate that is normally present in large amounts, suggests that the acyltransferase had not undergone a change in specificity.

A modification in the proportions of the products of the two unsaturated biosynthetic pathways was the major consequence at the 2-carbon with sterol substitution. Derivatives of the palmitoleic acid pathway increased at the expense of unsaturated products arising from stearate. A reduction in chain length and unsaturation resulted from this primary effect. The transformation in fatty acid patterns at the 2-carbon of these phospholipids also argues against a change in acyltransferase specificity due to the presence of sterol. No real change in enzyme reactivity in regard to chain length was evident. While overall chain length was reduced at the 2-carbon due to an enhancement of palmitoleic components and reduction in stearate derivatives, an increase in an 18-carbon acid, cilienic acid, also was seen. In addition, no alteration in specificity in terms of double bond location was evident. Fatty acids with Δ^6 double bonds both increased (18:$2\Delta^{6,11}$) and decreased (18:$3\Delta^{6,9,12}$), acyl groups with Δ^9 double bonds also increased (16:$1\Delta^9$) and decreased (18:$2\Delta^{9,12}$). The enzyme that acts at the 2-position appeared to substitute one class of unsaturates for another.

Since no apparent impact on the specificity of the acyltransferases was observed, an influence of ergosterol on fatty acid biosynthesis or on a differential turnover of esterified fatty acids might be anticipated. The first case would propose that the sterol supplementation leads to a quantitative change in the availability of the various fatty acids for incorporation into phosphorus-containing lipids. For example, one can envision that palmitate elongation to stearate or stearate desaturation is partially suppressed when ergosterol is the major membrane sterol. This could lead to the production of larger quantities of palmitoleic acid and its derivatives, as well as to an increase in short chain length acids by β-oxidation of palmitate or stearate. The second situation, on the other hand, would assume no change in fatty acid availability, but rather, an increased rate of deacylation and subsequent loss of those fatty acids belonging to the stearate pathway as compared to the palmitoleic acid pathway components. Further work is in progress to evaluate these two proposals.

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