Temperature-induced changes in the hydroxy and non-hydroxy fatty acid-containing sphingolipids abundant in the surface membrane of *Tetrahymena pyriformis* NT-1

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Abstract Sphingolipids make up 30 to 40 mole % of the phospholipids found in the surface membrane of *Tetrahymena pyriformis* NT-1. We have identified the two major classes as non-hydroxy fatty acid-containing ceramide-2-aminoethylphosphonate (NCAEP) and α-hydroxy fatty acid-containing ceramide-2-aminoethylphosphonate (HCAEP). Both classes were well represented in cells grown at 39°C. At this temperature their principal long chain bases were n-hexadeca-4-sphingenine and n-nonadeca-4-sphingenine. The major fatty acid of NCAEP from 39°C-grown cells was palmitic acid and that of HCAEP was α-hydroxypalmitic acid. Cells grown at 15°C contained NCAEP, but only traces of HCAEP. By analyzing the incorporation of [1-^{14}C]palmitic acid into cells growing isothermally or shifted from 15°C to 39°C, we obtained evidence favoring a direct conversion of NCAEP to HCAEP. This conversion was blocked in cells grown at 15°C, causing an accumulation of NCAEP. *Tetrahymena* is a useful model system for studying the poorly understood α-hydroxylation process that is of critical importance in myelination of animal nervous tissues.—**Kaya, K., C. S. Ramesha, and G. A. Thompson, Jr.** Temperature-induced changes in the hydroxy and non-hydroxy fatty acid-containing sphingolipids abundant in the surface membrane of *Tetrahymena pyriformis* NT-1. *J. Lipid Res.* 1984. **24:** 68–74.

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The occurrence of sphingolipids in the ciliated protozoan *Tetrahymena pyriformis* has been reported by several groups (1–6). Earlier work from this laboratory (6) has shown that sphingolipids constitute about 30–40% of the phospholipids in surface membranes of *Tetrahymena pyriformis* strain NT-1. Two major classes of sphingolipids were detected in the ciliary membranes. Based on their co-migration with authentic samples on TLC, these two sphingolipids were tentatively identified as ceramidephosphorylethanolamine (CPE) and ceramideaminoethylphosphonate (CAEP). The ratio of these two sphingolipids in cilia was drastically altered by growth temperature. Thus, "CPE" constituted about 23% of the ciliary phospholipids of 39°C-grown cells, while "CAEP" was present only in low levels. On the other hand "CAEP" constituted about 35% of the ciliary phospholipids of 15°C-grown cells, while "CPE" was virtually absent. The difference in the ratios of these two sphingolipids in the ciliary membranes of 39°C- and 15°C-grown cells correlates with the difference in physical properties of the ciliary membranes from 39°C- and 15°C-grown cells (6). Thus, it is possible that the sphingolipids play a prominent role in the temperature acclimation of the surface membranes. For this reason, it seemed desirable to characterize further the sphingolipids from 39°C- and 15°C-grown cells and their cilia.

A detailed characterization of the polar head groups and fatty acid and long chain base compositions of the two major sphingolipids found in *T. pyriformis* strain NT-1 is presented here. The structures of these two components are shown to differ in some respects from these indicated by the initial studies. Data favoring a direct metabolic relationship between these two sphingolipids are also described.

Abbreviations: CPE, ceramidephosphorylethanolamine; CAEP, ceramideaminoethylphosphonate; SP_1, low R_f sphingolipid; SP_2, high R_f sphingolipid; AEPL, 2-aminoethylglycerophosphonolipid; TMS, trimethylsilyl; LCB, long chain base; NCAEP, non-hydroxy fatty acid-containing ceramide-2-aminoethylphosphonate; HCAEP, α-hydroxy fatty acid-containing ceramide-2-aminoethylphosphonate; TLC, thin-layer chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry.

MATERIALS AND METHODS

Materials

[1-^{14}C]Palmitic acid (sp act 56.6 mCi/mmol) was purchased from New England Nuclear, Boston, MA. Saturated and unsaturated normal fatty acid methyl esters were obtained from Applied Science Laboratories, Col-

lege Station, PA. Bovine brain ceramide, phosphorylethanolamine, N-O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) were purchased from Sigma Chemical Co., St. Louis, MO. The silylating reagent (Silon HTP) was purchased from Supelco, Bellafonte, PA. Long chain bases were obtained by hydrolyzing bovine brain ceramides (7) and 2-aminoethylphosphonic acid was a gift from Dr. A. F. Isbell, Texas A&M University. Authentic samples of ceramide-2-aminoethylphosphonate and ceramidephosphorylethanolamine from molluscs were a gift from Dr. O. Itasaka, Shiga University, Otsu, Japan. All the other chemicals and solvents were of analytical grade.

Culture conditions

Tetrahymena pyriformis strain NT-1 was grown in 2% proteose peptone medium as described earlier (8). The cells were grown isothermally either at 39°C or at 15°C and were harvested in their midlogarithmic phase of growth (2–3 × 10^5 cells/ml).

Isolation of cilia

Cells were deciliated by the Ca^{2+} shock procedure (9), and the cilia were purified using the procedure of Ramesha and Thompson (10).

Lipid analysis

Lipids were extracted from isolated cilia and cell bodies by the procedure of Bligh and Dyer (11). Total lipid phosphorus was estimated by the method of Bartlett (12) as modified by Marinetti (13). The distribution of individual phospholipid classes as determined by the method of Rouser, Fleischer, and Yamamoto (14).

Purification of sphingolipids

Sphingolipids from ciliary and whole cell lipids were purified as follows. The total lipid fraction was subjected to mild alkaline hydrolysis at room temperature for 1 hr in methanolic 1.5 N sodium hydroxide (4). The alkali-stable lipids were extracted with chloroform and taken to dryness under a stream of N_2. The lipid residue was dissolved in a small volume of chloroform–methanol 6:1 (v/v), applied to a preparative silica gel H plate (0.5-mm thickness), and developed in chloroform–methanol–water 65:30:5 (v/v). After briefly exposing the plate to iodine vapor, two major sphingolipid bands (referred to hereafter as SP_1, R_f 0.48; and SP_2, R_f 0.56) were scraped off the plate and eluted with chloroform–methanol–water 1:6:0.4 (v.v). After concentrating SP_1 and SP_2, they were rechromatographed on a silica gel H plate using chloroform–methanol–ammonia 65:25:5 (v/v) as the solvent. In this solvent system, SP_1 showed one major band and four minor bands, while SP_2 showed one major band and three minor bands. The four minor bands contaminating SP_1 were lysoAEPL, SP_2, and two non-phosphorus-containing ceramide lipids, while three minor bands found with SP_2 were SP_1 and two non-phosphorus-containing ceramide lipids. No bands co-migrating with either ceramidephosphorylethanolamine or ceramide-N-methylaminoethylphosphonate standards were observed. The major bands, SP_1 and SP_2, were eluted and purified further on 2% boric acid-impregnated silica gel H plates using chloroform–methanol–ammonia 65:25:5 (v/v) as the solvent. Both SP_1 and SP_2 thus isolated were utilized for further structural analysis.

Polar head group analysis

The following methods were used to identify the polar head groups of SP_1 and SP_2.

Analysis of the phosphorus linkage. A determination of the proportion of lipid-bound phosphorus in phosphodiester and phosphonic acid ester form was made as follows. Both SP_1 and SP_2 were hydrolyzed separately with 10 N H_2SO_4 for the hydrolysis of ester phosphorus and with 70% perchloric acid for hydrolyzing total phosphorus. The difference between total phosphorus and ester phosphorus was taken as the amount of phosphonate phosphorus (15).

Analysis of water-soluble products. Both SP_1 and SP_2 were hydrolyzed with 6 N HCl at 110°C for 24 hr (16). The water-soluble compounds were concentrated to dryness under nitrogen and further dried in a vacuum desiccator over sodium hydroxide pellets. The residue was analyzed by paper chromatography, thin–layer chromatography, and gas–liquid chromatography (GLC).

Paper chromatography. Aliquots of the water-soluble products of SP_1 and SP_2 hydrolysis were spotted on Whatman No. 1 filter paper strips along with standards, including 2-aminoethylphosphonic acid and phosphorylethanolamine, and developed overnight in n-butanol–acetic acid–water 4:2:1 (v/v). After drying the paper, the spots were made visible by spraying with ninhydrin (or ammonium molybdate for phosphorus). R_f values for standards include: 2-aminoethylphosphonic acid, 0.18; ethanolamine, 0.32; inorganic phosphate, 0.21; phosphorylethanolamine, 0.15.

Thin-layer chromatography. Another aliquot of the water-soluble products of SP_1 and SP_2 hydrolysis was spotted on a silica gel G plate with standard 2-aminoethylphosphonic acid. The plate was developed in 72% phenol–ethanol–acetic acid 50:5:6 (v/v) (17). After drying the plate, the spots were visualized with ninhydrin or the silica gel was cut into different bands and their phosphorus content was estimated by the procedure of Rouser et al. (14). 2-Aminoethylphosphonic acid migrated with an R_f of 0.20.

Gas–liquid chromatography. A third aliquot of the water-soluble residue of SP_1 and SP_2 hydrolysis was converted

to trimethylsilyl (TMS) derivatives by reacting with BSTFA–pyridine–TMCS (10:5:2, by vol) at 100°C for 1 hr (16). The TMS derivatives were analyzed by gas–liquid chromatography.

Long chain base composition

The long chain base (LCB) composition of SP_1 and SP_2 was determined as follows. SP_1 and SP_2 were hydrolyzed with methanol–12 N HCl 5:1 (v/v) at 75°C for 18 hr (4). After extracting the fatty acid methyl esters, the pH of the aqueous phase was brought to 11 with 1 N sodium hydroxide, and the LCB's were extracted, acetylated, and converted to N-acetyl-O-TMS derivatives by reacting with Silon HTP as described by Tao et al. (18). The N-acetyl-O-TMS derivatives were then analyzed by GLC using N-acetyl-O-TMS LCB prepared from bovine brain ceramides as standards (7).

Fatty acid analysis

The fatty acid methyl esters extracted as described in the preceeding paragraph were taken to dryness under a stream of nitrogen and were acetylated by the method of Totani and Muramatsu (19). The acetylated fatty acid methyl esters were then analyzed by GLC.

Incorporation of [1-^{14}C]palmitic acid into sphingolipids

Incorporation of [1-^{14}C]palmitic acid into SP_1 and SP_2 was carried out as follows. *Tetrahymena* growing either at 39°C or at 15°C were harvested, and the cell pellet was washed twice with incubation buffer at the cells' growth temperature (phosphate buffer, 1 mM, pH 6.8, containing 100 nM $MgSO_4$, 4.75 mM NaCl, 2 mM glucose, and 45 μM iron-EDTA complex). The washed cell pellet was gently suspended in the incubation buffer (at either 39°C or 15°, as required) to a final density of 2–3 × 10^5 cells/ml. [1-^{14}C]Palmitic acid (0.5 μCi) in 40 μl of ethanol was immediately added into the flask and the cells were incubated either at 39°C or at 15°C. Essentially all of the added fatty acids were esterified into complex lipids by 10 min at 39°C, or 30 min at 15°C. At specified intervals, aliquots of the cells were taken, their lipids were extracted, and sphingolipids were separated. Fatty acid methyl esters of the sphingolipids were prepared, and the radioactivity in hydroxy and non-hydroxy fatty acid methyl esters was determined after separating the ester classes by TLC using n-hexane–diethylether 85:15 (v/v) as the solvent.

Gas–liquid chromatography (GLC)

GLC analysis was done on a Varian Model 3700 chromatograph equipped with a flame ionization detector.

The acetylated fatty acid methyl ester mixture and the TMS derivatives of the polar head groups were analyzed using a 10 m × 0.25 mm i.d. open tubular glass column coated with SP2330 (Supelco, Bellafonte, PA.). The column temperature was maintained at 170°C and nitrogen head pressure was set at 0.4 kg cm^{-2}. Injector and detector temperatures were both held at 230°C. Split injection (split ratio of 40:1 to 100:1) was used. The N-acetyl-O-TMS derivatives of the long chain bases were analyzed by using a packed 3% OV-101 glass column (6 ft × 0.25 in i.d.) at 220°C. Injector and detector temperatures were maintained at 250°C and 270°C, respectively.

The identity of the sphingolipid fatty acids as being the C_{16} and C_{17} species was confirmed by GLC–MS, using conditions described previously (20). The absence of fatty acid unsaturation was demonstrated by GLC analysis before and after catalytic hydrogenation over platinum. Tentative identification by GLC of the minor LCB components, including heptadeca-, octadeca-, and nonadeca-4-sphingenine, was also confirmed by GLC–MS.

RESULTS

Total sphingolipids were prepared from cilia and cells of *Tetrahymena* grown at 39°C and 15°C by alkaline hydrolysis of the total lipids. The two individual sphingolipid classes were then further purified by successive TLC (see Methods for details). Thus purified, the two classes, designated SP_1 (having the lower TLC mobility) and SP_2 (having the higher TLC mobility) were utilized for structural analyses.

Analysis of the polar head group

Analysis for the presence of a direct carbon-phosphorus linkage (C-P linkage) indicated that both SP_1 and SP_2 are phosphonate derivatives (**Table 1**). Further characterization of the polar head group was done by first hydrolyzing SP_1 and SP_2 with HCl and then analyzing the water-soluble compounds by paper chromatography, TLC, and GLC. The water-soluble hydrolysis products of both SP_1 and SP_2 showed a ninhydrin-positive band on paper which co-migrated with an authentic 2-aminoethylphosphonic acid standard and differed from other potential products (see Methods). Similarly, the hydrolysis products both gave a ninhydrin-positive band on TLC which co-migrated with 2-aminoethylphosphonic acid. More than 90% of the phosphorus present in the water-soluble hydrolysis products was recovered in the ninhydrin-positive band scraped from TLC plates.

Further proof of the polar head group identity was obtained by GLC analysis. The TMS derivatives of the polar head groups of both SP_1 and SP_2 had the same retention time as that of 2-aminoethylphosphonic acid (retention time 0.5 min); there were no peaks corresponding to phosphorylethanolamine or phosphoryl-N-methylethanolamine, whose retention times, relative to

TABLE 1. Nature of the carbon–phosphorus linkage in specific *Tetrahymena* ciliary phospholipids

Class of Phospholipid	% of Total Phosphorus as Phosphonate Phosphorus	% of Total Phosphorus as Ester Phosphorus
SP_1	93	7
SP_2	97	3
Phosphatidylethanolamine	6	94
2-Aminoethylphosphonolipid	99	1

Values are the average of two independent determinations. Details of phosphorus analyses are given in Materials and Methods. SP_2 was isolated from 15°C-grown cells and the other three lipids from 39°C-grown cells (polar head groups of each lipid class were shown to be identical at the two growth temperatures).

2-aminoethylphosphonic acid, are 1.5 and 0.4. Thus, from the above results, it is clear that both SP_1 and SP_2 are 2-aminoethylphosphonolipids.

Long chain base composition of SP_1 and SP_2

Since both SP_1 and SP_2 had the same polar head group, the difference in TLC mobility must be attributed to their long chain base (LCB) composition or their fatty acid composition.

Analysis of the N-acetyl-O-TMS derivative of the LCB bases revealed a similar distribution for both SP_1 and SP_2 of 39°C-grown cells (**Table 2**). Both of them contained two major LCB's, n-hexadeca-4-sphingenine and n-nonadeca-4-sphingenine, which were present in almost equal proportions (each about 45% of the total). The LCB composition of SP_2 from 15°C-grown cells was basically similar to that of 39°-grown cells, except that n-hexadeca-4-sphingenine was the major LCB (about 50% of the total) and iso-heptadeca-4-sphingenine and n-nonadeca-4-sphingenine each constituted about 18% of the total. Since SP_1 was present in only trace amounts in 15°C-grown cells, it was not analyzed. The LCB compositions of whole cell sphingophosphonolipids from 39°C- and 15°C-grown *Tetrahymena* were very nearly identical to those of their respective ciliary membranes (Table 2).

Fatty acid composition of SP_1 and SP_2

Table 3 shows the fatty acid composition of SP_1 and SP_2 from 39°C- and 15°C-grown *Tetrahymena*. The major difference between SP_1 and SP_2 was in their fatty acid compositions (**Fig. 1,** also Table 3). SP_1 from 39°C-grown *Tetrahymena* contained mainly hydroxy fatty acids while SP_2 from both 39°C- and 15°C-grown *Tetrahymena* contained mainly non-hydroxy fatty acids. The major fatty acids of SP_2 from 15°C-grown cells were 16:0 and iso-17:0 while the principal fatty acid of SP_2 from 39°C-grown cells was 16:0. The major hydroxy fatty acid of SP_1 from 39°C-grown cells was hydroxy 16:0. The fatty acid compositions of SP_2 from 15°C-grown cells and cilia were similar as were those of SP_1 from 39°C-grown cells and cilia. SP_2 from 39°C-grown cells and cilia showed some differences in their fatty acid compositions (Table 3). Further experimentation showed that the small amounts of hydroxy fatty acids observed in SP_2 of 39°C-grown cells was due to a slight contamination with SP_1. Likewise, continued purification of SP_1 resulted in a loss of non-hydroxy fatty acids, while the relative proportions of each hydroxy fatty acid remained constant.

The data described above, taken together, prove conclusively the identity of SP_1 as hydroxy-fatty acid-containing ceramideaminoethylphosphonates (HCAEP). By the same criteria, SP_2 is a class of ceramideaminoethylphosphonates (NCAEP) in which the N-acyl components are non-hydroxy fatty acids.

Biosynthesis of NCAEP and HCAEP in 39°C- and 15°C-grown *Tetrahymena*

Because of the striking difference in the relative proportions of these two closely related sphingolipid classes in 39°C- and 15°C-grown cells, basic data on their biosynthesis was sought by monitoring the incorporation of

TABLE 2. Long chain base (LCB) composition of sphingolipids in whole cells and cilia of *Tetrahymena pyriformis* NT-1

Long-Chain Base	15°C-Grown Cells SP_2 Whole Cell	15°C-Grown Cells SP_2 Cilia	39°C-Grown Cells SP_1 Whole Cell	39°C-Grown Cells SP_1 Cilia	39°C-Grown Cells SP_2 Whole Cell	39°C-Grown Cells SP_2 Cilia
n-Hexadeca-4-Sph	54.9	46.9	45.7	44.1	44.6	41.7
iso-Heptadeca-4-Sph	18.9	19.4	0.2	tr	0.2	tr
n-Heptadeca-4-Sph	3.2	5.2	5.7	6.8	6.2	5.6
iso-Octadeca-4-Sph	1.4	tr	0.3	tr	0.7	tr
n-Octadeca-4-Sph	3.8	4.7	3.2	5.0	4.1	3.9
iso-Nonadeca-4-Sph	2.3	6.2	1.9	tr	2.2	2.6
n-Nonadeca-4-Sph	15.4	17.5	43.0	44.1	42.0	46.2

Values are expressed as weight % of total. tr = trace (<0.1%). Details of isolation and GLC analysis of long chain bases are given under Methods.

TABLE 3. Fatty acid composition of sphingolipids from whole cells and cilia of *T. pyriformis* NT-1

	15°C-Grown Cells		39°C-Grown Cells			
	SP_2		SP_1		SP_2	
Fatty Acid	Whole Cell	Cilia	Whole Cell	Cilia	Whole Cell	Cilia
14:0	0.4	0.4	0.5	1.8	1.5	18.4
15:0	0.2		0.2	0.5	0.8	2.5
16:0	57.1	57.0	10.0	5.8	70.9	44.3
i-17:0[a]	40:2	39.6	0.5	1.2	8.6	14.2
17:0	0.7	2.1	0.9	0.9	3.8	3.8
i-18:0	0.6	0.2				
18:0	0.2	0.5	0.9	0.6	6.6	5.5
i-19:0				0.8	1.1	5.7
16h:0[b]	0.5	0.2	63.8	71.6	4.9	5.7
i-17h:0	0.1		9.9	7.5	0.8	
17h:0			7.6	5.4	0.6	
18h:0			5.7	3.9	0.4	
Non-hydroxy:hydroxy fatty acid ratios						
	99.4:0.6	99.8:0.2	13.0:87.0	11.6:88.4	93.3:6.7	94.3:5.7

Values are expressed as weight % of total peak area and are typical of data from several replicate experiments. Details of isolation and GLC analysis of the fatty acids are given under Methods.

[a] i, indicates *iso* branched fatty acid.

[b] h, indicates hydroxy fatty acids.

[1-^{14}C]palmitic acid into both lipids. Basically, these are pulse-labeling experiments, since virtually all the added free fatty acids are taken up by the cells and incorporated into complex lipids within 10–15 min. 39°C-Grown cells when incubated at 39°C incorporated significant amounts of radioactivity into their sphingolipids. The radioactivity present in fatty acids of the two sphingolipid classes could be accurately estimated by hydrolyzing the purified total sphingolipid fraction and separating the non-hydroxy fatty acids (from NCAEP) and the hydroxy fatty acids (from HCAEP). Incorporation of the radioactivity into non-hydroxy and hydroxy fatty acids was equal during the first hour of incubation (**Fig. 2**). From then on there was no net increase in normal fatty acid radioactivity. On the other hand, the incorporation into hydroxy fatty acids increased after the first hour and continued in a linear fashion for at least 4 hr.

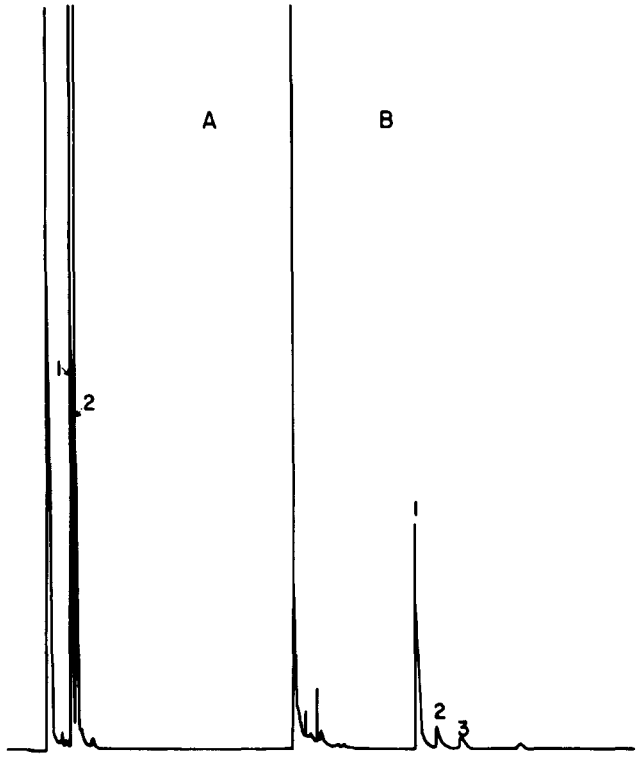

Fig. 1. Gas chromatogram of fatty acid methyl esters. A. From SP_2, 16:0 (1) and iso 17:0 (2). B. From SP_1, hydroxy 16:0 (1), hydroxy iso 17:0 (2), and hydroxy 17:0 (3). Details of GLC are given in the text.

Incorporation of radioactivity into sphingolipids of 15°C-grown cells at 15°C was about 3 times lower than that into sphingolipids of 39°C-grown cells, and most of the radioactivity was in non-hydroxy fatty acids. No radioactivity was observed in the hydroxy fatty acid fraction of these lipids even after 4 hr of incubation (data not shown).

When 15°C-grown cells were quickly (5 min) raised to 39°C and incubated at that temperature with [1-^{14}C]palmitate, there was an immediate and substantial appearance of radioactivity in the non-hydroxy fatty acid components of the sphingolipids (**Fig. 3**). After 2 hr the rise of nonhydroxy fatty acid radioactivity ceased and was supplanted by a delayed increase in [1-^{14}C]hydroxy fatty acids. Preliminary results have indicated that in the first few hours after warming 15°C-grown cells to 39°C, the absolute quantity of NCAEP present in the cells grad-

ually decreases and is offset by an equal increase in the amounts of HCAEP. All these data strongly indicate a precursor-product relationship between NCAEP and HCAEP and show that the synthesis of HCAEP is greatly inhibited by low temperature.

DISCUSSION

We reported earlier the presence of two major classes of sphingolipids in ciliary membranes of *Tetrahymena* grown at 39°C and 15°C (6). The detailed analysis of the polar head group components of the purified sphingolipid classes presented here clearly demonstrates that both are 2-aminoethylphosphonate derivatives. Taking into account the long chain base and fatty acid analyses, we have now confirmed that the chromatographically slow moving component (SP_1) is an α-hydroxy fatty acid-containing ceramideaminoethylphosphonate (HCAEP) and the faster moving component (SP_2) is a non-hydroxy fatty acid-containing ceramideaminoethylphosphonate (NCAEP).

Identification and partial characterization of sphingolipids in *Tetrahymena* have previously been reported by other workers (1–5). The polar head group and fatty acid composition and also the relative distribution of different sphingolipid classes seem to vary with the strain of *T. pyriformis* used and also the culture conditions employed. Sugita et al. (4) have carried out a detailed structural analysis of sphingolipids of *T. pyriformis* strain WH-14. The major sphingolipid classes and the long chain base and fatty acid compositions of the strain WH-14 are different from those reported here for strain NT-1. Our demonstration that the formation of HCAEP is dependent upon the cells' growth temperature may explain some of the inconsistencies in the findings of various laboratories.

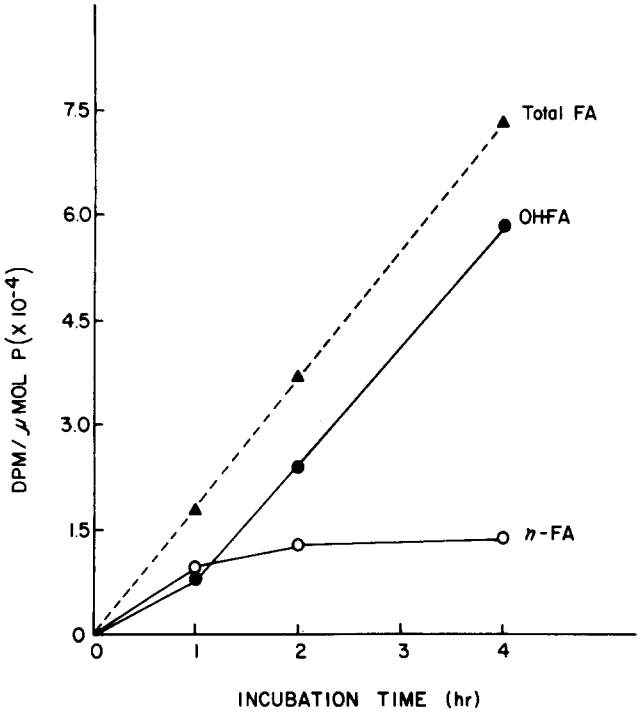

Fig. 2. Incorporation of [1-^{14}C]palmitic acid into the fatty acids of sphingolipids of 39°C-grown cells. The radioactive fatty acid was added at time zero, and the values represent the radioactivity present in total fatty acids (total FA), hydroxy fatty acids (OH-FA) and non-hydroxy fatty acids (n-FA) Details of incubation and analysis of the radioactivity are described in the text.

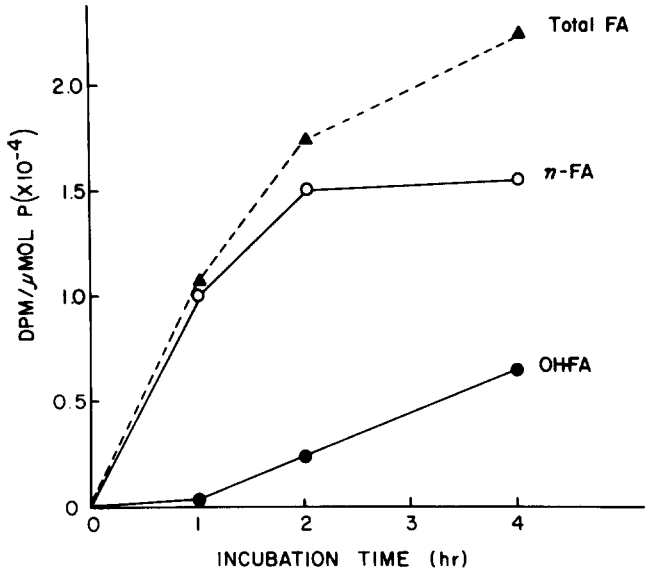

Fig. 3. Incorporation of [1-^{14}C]palmitic acid into the fatty acids of sphingolipids of 15°C-grown cells shifted to 39°C. The radioactive fatty acid was added at time zero following the shift. Remaining details are same as in Fig. 2.

While the value of the ceramideaminoethylphosphonates to their parent cell is still conjectural, the entire system has another more practical value. *Tetrahymena* can serve as a model system for studying the process of fatty acid α-hydroxylation. Despite the importance of α-hydroxylation as a regulatory factor in central nervous system myelination (21, 22), the molecular mechanisms involved are still not known. In work now underway[1] we have obtained strong evidence favoring a direct hydroxylation of ceramide-bound normal fatty acids rather than the presently favored pathway (23, 24) calling for the hydroxylation of free fatty acids prior to their incorporation into sphingolipids.

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