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CORRELATION BETWEEN FLUIDITY AND FATTY ACID COMPOSITION OF PHOSPHOLIPID SPECIES IN *TETRAHYMENA PYRIFORMIS* DURING TEMPERATURE ACCLIMATION

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Summary

The correlation between the fluidity of phospholipids and their fatty acid composition was studied by spin label technique and gas-liquid chromatography for three major phospholipid species in *Tetrahymena pyriformis* during temperature acclimation. The fluidity of 2-aminoethylphosphonolipid increased within the first 10 h of the cold-acclimation when the content of γ-linolenic acid in 2-aminoethylphosphonolipid was highest, and it then decreased up to 24 h. On the other hand, the fluidities of phosphatidylethanolamine and phosphatidylcholine showed a gradual decrease up to 24 h after the temperature shift, although γ-linolenic acid contents were highest at 10 h after the temperature shift. Thus the fluidity changes of these two phospholipids were interpreted as resulting from the altered content of other fatty acids in addition to γ-linolenic acid, since the γ-linolenic acid content was smaller than that of 2-aminoethylphosphonolipid. The results suggest that the content of γ-linolenic acid in 2-aminoethylphosphonolipid plays a role in regulating the thermal adaptation process.

Introduction

It is well known that living cells alter their lipid composition to maintain membrane fluidity in both prokaryotes [1—4] and eukaryotes [5,6]. Membrane fluidity and lipid composition play an important role for the function of biological membranes. Recently the regulation of membrane enzymes by lipids was reviewed by Sandermann [7]. The ciliate *Tetrahymena pyriformis*, an eukaryotic cell, which has highly developed subcellular organelles similar to

mammalian cells [8], was also observed to change the fatty acid composition of its membranes when chilled to a lower temperature [9]. We have shown in subcellular organelles of *T. pyriformis* that the proper fluidity for membrane function is maintained in the new temperature environment by changing the fatty acid composition, especially the degree of its unsaturation [10]. We have then extended the study to obtain information about two points; regulatory mechanism of desaturating fatty acid chains of phospholipids and effects of altered lipid composition on membrane fluidity under the condition that the cells are chilled to a lower temperature. Evidence was provided that the mechanism of thermal adaptation may be due rather to the elevation of the level of palmitoyl CoA desaturase than to its direct control by membrane fluidity per se [11,12]. Also we have observed that there is a correlation between the physical property of membrane examined by the pattern of intramembrane particles and the lipid composition during the temperature acclimation [13]. The alterations of fluidities of membranes and its extracted lipids were reported to occur during temperature acclimation using fluorescence probe by Martin and Thompson [14]. Various membranes of *Tetrahymena* comprise three major phospholipids. It is presumed that phospholipid plays an important role in the adaptation mechanism. However, little or no information has been available regarding the involvement of phospholipid head groups in modulating membrane fluidity for thermal adaptation. In the present paper, we report the results of a study of correlation between the change in fluidity and fatty acid composition of each phospholipid class from *Tetrahymena* cells during the adaptation to a lower growth temperature.

Materials and Methods

Cell culture

The cells of a thermotolerant strain of *Tetrahymena pyriformis* NT-1 were grown in an enriched medium at 39.5°C. The cell density was measured with a hemocytometer. Cells in the log growth phase were used in all experiments.

Conditions for temperature shift

All procedures were carried out according to the previous paper [12]. Cells for the temperature shift-down experiment were grown in 200 ml medium at 39.5°C to a cell density of approx. $1.5—2.0 \cdot 10^5$ cells/ml culture, and were cooled to 15°C for over 30 min by swirling the growth flask in an ice-water slurry. The rate of cooling was essentially linear (0.8°C/min) and was monitored by placing a sterile thermometer directly into the medium. After cells were cooled to 15°C, cell division did not occur for approx. 10 h. However, after this period of time cells began to grow with a generation time of 10 h.

Lipid composition analysis

Lipid extraction was carried out according to Bligh and Dyer [15] and the resultant lipids were stored in $CHCl_3/CH_3OH$ (6 : 1, v/v) at —20° C.

Phospholipids were separated from neutral lipids by silicic acid/hyflo-supercel column chromatography [16]. Phospholipids were separated on a Silica gel H thin-layer chromatographic plate, and the corresponding spots of individual

phospholipids were scraped off the plate. Quantitative analysis of fatty acids was carried out by gas-liquid chromatography as previously described [13]. Unsaturation index was defined as Σ (% content of unsaturated fatty acids) × (number of double bond).

ESR spectroscopy

The stearate spin probe, *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-ketostearic acid, was synthesized according to the procedure of Waggoner et al. [17]. For preparation of spin-labeled phospholipid dispersion, the lipid extract in chloroform/methanol was mixed with 1 mol% of the spin probe in benzene and the solvent was evaporated first in a nitrogen stream and then by evacuation. Then 0.5 ml of Tris-buffered saline (150 mM NaCl and 50 mM Tris-HCl, pH 7.5) and several small glass beads were added and vortexed with a Thermomixer (Thermonics Co. Ltd.) above 40°C for 1 min. The suspension was sonicated for 30 s with a 20 kHz sonifier (Kaijo Denki, Co. Ltd.) fitted with a titanium tip (3 mm diameter) at a power level of 10. As soon as the sonication was stopped, the suspensions of phosphatidylethanolamine and 2-aminoethylphosphonolipid became turbid. The vesicles were collected by centrifugation at 15 000 × *g* for 10 min, and used for ESR measurements. Phosphatidylcholine suspension was completely homogeneous and was then used for ESR measurements without centrifugation.

The spin-labeled dispersion of extracted lipids was taken into a cylindrical quartz tube and ESR spectra were measured at various temperatures using a commercial X-band spectrometer (JEOL FE-2X) equipped with a variable temperature control. The rate of temperature decrease was no greater than 0.5°C per min. The parallel ($T_{\parallel}$) and perpendicular ($T_{\perp}$) principal values of the hyperfine tensor of an axially symmetrical spin Hamiltonian were estimated from the ESR spectra and the order parameter S was calculated using the relation $S = (T_{\parallel} - T_{\perp})/(T_{zz} - T_{xx})$ where T_{zz} (32.9 G) and T_{xx} (5.9 G) are the hyperfine principal values of the nitroxide radical.

Results

T. pyriformis cells contain three major phospholipids, phosphatidylethanolamine, phosphatidylcholine and 2-aminoethylphosphonolipid. The 2-aminoethylphosphonolipid is an unusual lipid having a carbon-phosphorus bond and is enriched in ether-linked hydrocarbon side-chains [18]. There was little significant alteration in phospholipid class composition within 10 h after the temperature shift (data not shown), which is consistent with the previous report [12].

It is well known that phosphatidylethanolamine forms hexagonal and lamellar structures [19,20]. In the present study, ethanolamine-containing phospholipids, phosphatidylethanolamine and 2-aminoethylphosphonolipid which have similar head group were found not to form the clear liposomes such as phosphatidylcholine by sonication. During sonication the suspension of phosphatidylethanolamine or 2-aminoethylphosphonolipid was rather clear and transparent, but became turbid after sonication was stopped. Fig. 1 shows the ESR spectra of stearate spin probe in 2-aminoethylphosphonolipid vesicles.

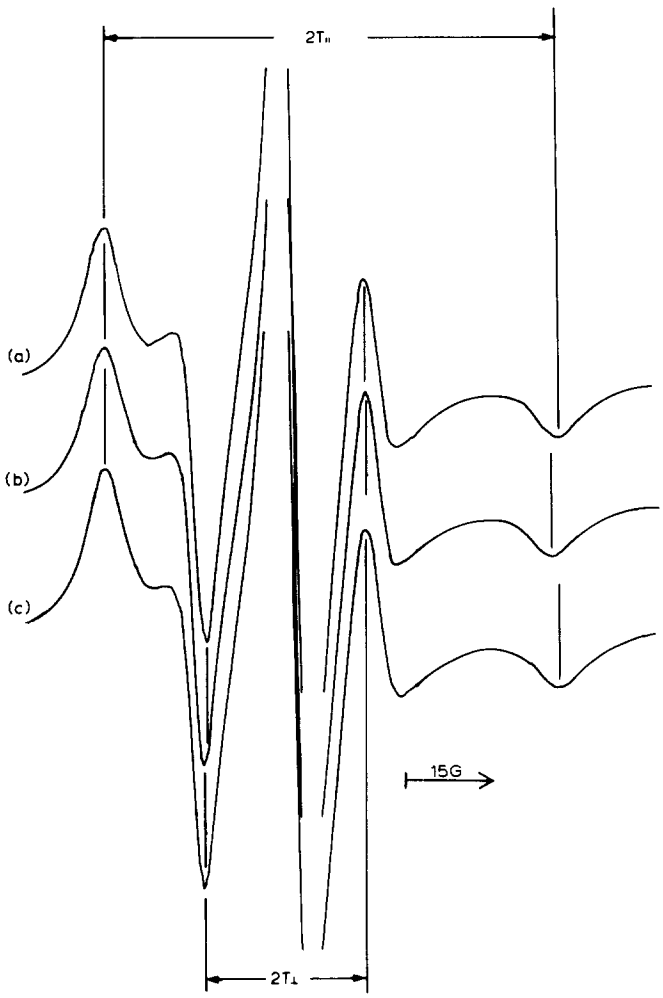

Fig. 1. ESR spectra of stearate spin probe at 25°C in 2-aminoethylphosphonolipid extracted from *Tetrahymena* cells at various times after the temperature shift. 2-Aminoethylphosphonolipid, separated from the lipid extracts of *Tetrahymena* cells (a) at 2 h, (b) at 10 h and (c) at 24 h after the temperature shift, was cosonicated with 1 mol% of stearate spin probe in phosphate-buffered saline. The lipid vesicles collected by centrifugation were used for ESR measurement. $2T_{\parallel}$ and $2T_{\perp}$ were measured as shown in the spectra.

These spectra indicate the anisotropic motion as observed with spin probes inserted in the lipid bilayer. There was no difference in the spectra between phospholipids with ehtanolamine group and phosphatidylcholine liposomes. The order parameter was calculated from $T_{\parallel}$ and $T_{\perp}$ obtained from ESR spectrum.

When *Tetrahymena* cells are quenched to a lower temperature, they change the physical state of the membranes [13] and their extracted lipids [14] to be more fluid with time. In order to know whether or not some specific phospholipid does contribute to adjusting the physical state of membranes to the new temperature, the time course of fluidity change was examined for each of three

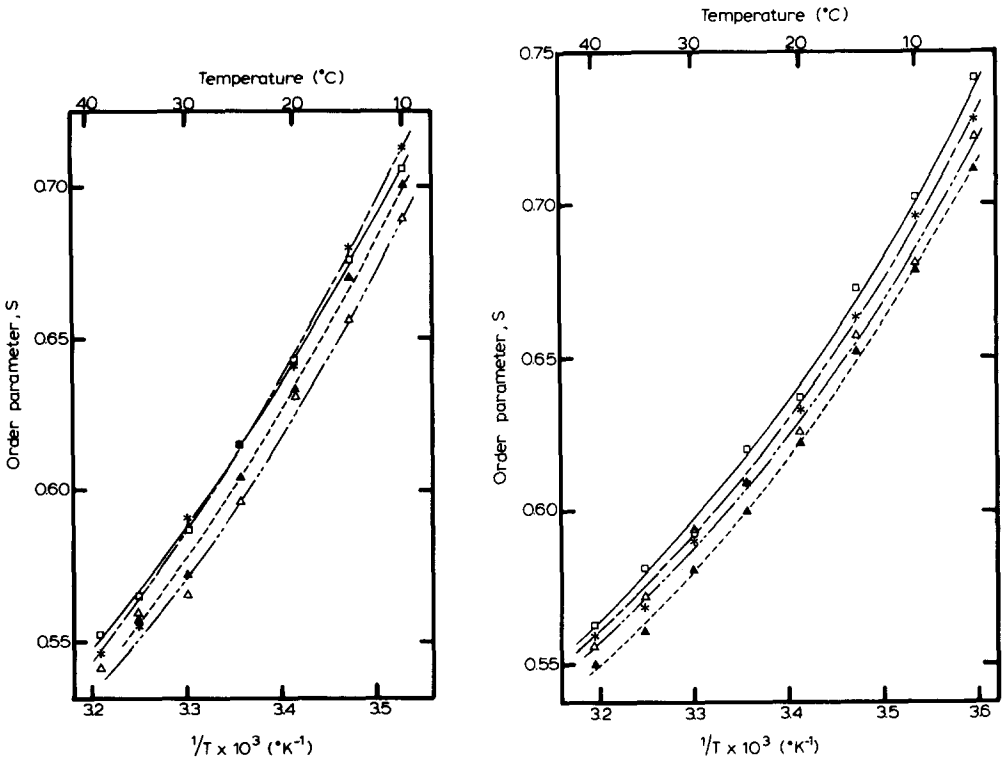

Fig. 2. The order parameter vs. temperature of 2-aminoethylphosphonolipid prepared at various intervals after the temperature shift. Sample preparation and ESR measurement are described in Fig. 1. Sample preparations were at 2 h (□———□), 4 h (★- — - — -★), 10 h (△- — - — -△) and 24 h (▲- - - - -▲) after the temperature shift.

Fig. 3. The order parameter vs. temperature of phosphatidylethanolamine prepared from 2 h (□———□), 4 h (★- — - — -★), 10 h (△- — - — -△) and 24 h (▲- - - - -▲) cells. Sample preparation and ESR measurement are the same as Fig. 1.

major phospholipids separated from the total lipid extracts of *Tetrahymena* cells.

Fig. 2 shows the plot of order parameter vs. temperature for vesicles of 2-aminoethylphosphonolipid prepared from the cells at various times after the temperature shift. Each curve represents the temperature dependence of order parameter demonstrating the increased fluidity of lipids at higher temperatures. No change in order parameter is observed within the first 4 h, and the maximum decrease is observed at 10 h after the temperature shift. Then the order parameter increased up to 24 h. It is thus indicated that the fluidity of 2-aminoethylphosphonolipid did not change for the first 4 h but increased markedly during the next 6 h and then decreased to the intermediate state between 2 (or 4) h and 10 h (see Fig. 1).

The changes in order parameter of phosphatidylethanolamine are plotted in Fig. 3. The order parameters were found to decrease in a parallel manner at all temperatures tested as time passed. Although the difference in order parameter is distinct between 2 and 24 h, there was a small but significant difference

between 4 and 10 h. These results indicated that phosphatidylethanolamine shows progressive increases in the fluidity with time after the shift to 15°C.

Adaptation of phosphatidylcholine to a lower temperature is also reflected as the decrease in order parameter, which is compatible with that of phosphatidylethanolamine (Fig. 4).

The fatty acid composition was examined for each phospholipid class at various times after the temperature shift (Table I). Some parameters are employed to characterize the fatty acid composition, for examples, the ratio of unsaturated to saturated fatty acid content, unsaturation index and the ratio of palmitoleate to palmitate content ($C_{16:1}/C_{16:0}$). The compositional change of fatty acid was mainly observed in palmitic ($C_{16:0}$) and γ-linolenic ($C_{18:3}$, $\Delta^{6,9,12}$) for all three phospholipids. The relative content of $C_{16:0}$ reduces gradually until 10 h after the shift, and then increased up to 24 h. In contrast, $C_{18:3}$ increased profoundly for the first 10 h and decreased thereafter. At 10 h after the temperature shift, $C_{16:0}$ content is lowest and $C_{18:3}$ content is highest in these three phospholipids.

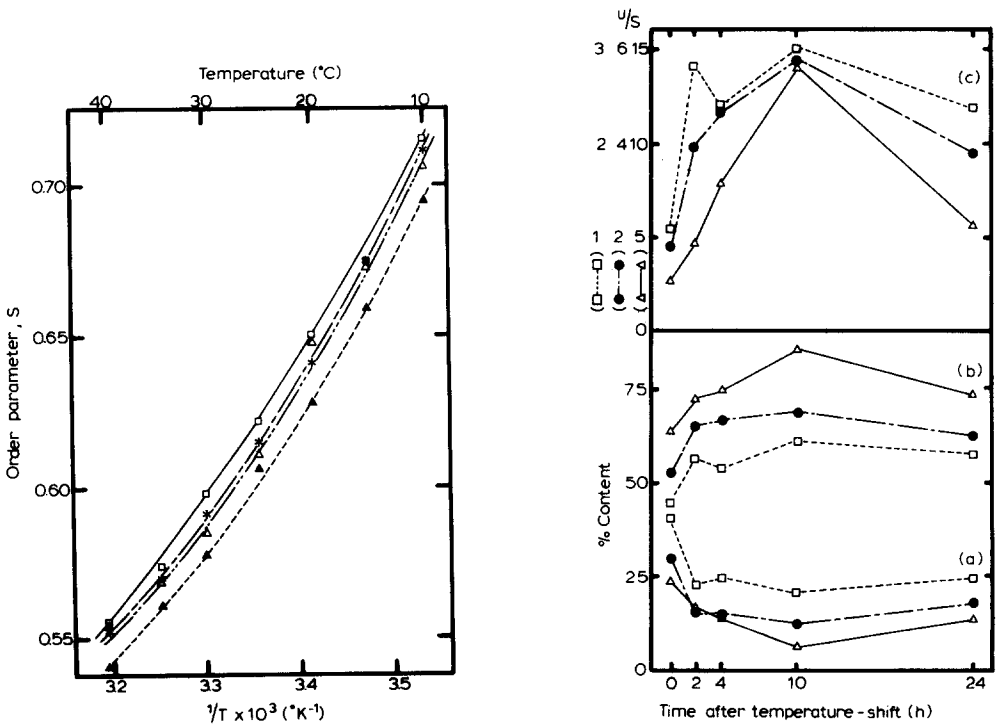

Fig. 4. The order parameter vs. temperature of phosphatidylcholine prepared from 2 h (□————□), 4 h (★- — — -★), 10 h (△— - - —△) and 24 h (▲- - - - - -▲) cells. Sample preparation and ESR measurement are the same as Fig. 1.

Fig. 5. Time course of the content of saturated (a) and unsaturated (b) fatty acids, and the ratio of unsaturated to saturated fatty acid (c). 2-Aminoethylphosphonolipid (△————△), phosphatidylethanolamine (□- - - - -□) and phosphatidylcholine (●- — — -●) were separated by thin-layer chromatography from the lipid extracts of *Tetrahymena* cells taken at 0, 2, 4, 10 and 24 h after the temperature shift. Analysis of the fatty acid composition was carried out by gas-liquid chromatography.

Fig. 5. shows the content of saturated (a) and unsaturated fatty acids (b) and the ratio of unsaturated to saturated fatty acid (c). All three phospholipids have the maximum level of unsaturated fatty acid content at 10 h and the general pattern of the plots seems rather similar to each other. The content of unsaturated fatty acid reflects mainly that of γ-linolenic acid which is richest in 2-aminoethylphosphonolipid and least in phosphatidylethanolamine. The

TABLE I

FATTY ACID COMPOSITION OF EACH PHOSPHOLIPID CLASS PREPARED FROM *TETRAHYMENA* CELLS DURING ADAPTATION TO 15°C

The values represent averages ± S.D. of 3 experiments except 0 h values (1 experiment). U/S: the ratio of unsaturated to saturated fatty acid. 16:1/16:0: the ratio of palmitoleic to palmitic acid.

Phospholipid	Time after the temperature shift (h)				
	0	2	4	10	24
2-Aminoethyl-phosphonolipid					
12:0	0.7	0.4 ± 0.3	—	—	0.2 ± 0.1
14:0	6.8	3.3 ± 1.5	3.1 ± 1.8	1.5 ± 0.5	4.7 ± 0.9
15:0 (iso)	2.6	1.3 ± 0.7	1.9 ± 0.1	0.8 ± 0.2	1.2 ± 0.2
15:0	1.6	0.7 ± 0.5	1.1 ± 0.4	0.5 ± 0.1	0.7 ± 0.2
16:0 (iso)	0.5	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
16:0	11.2	10.8 ± 4.2	8.5 ± 4.2	3.3 ± 0.3	6.3 ± 0.4
16:1	12.7	9.2 ± 1.8	12.0 ± 2.5	7.3 ± 0.4	11.8 ± 0.5
16:2 + 17:0	5.6	4.4 ± 1.2	5.6 ± 0.7	5.4 ± 0.4	6.1 ± 0.4
unknown	1.6	0.9 ± 0.3	1.5 ± 0.6	1.3 ± 0.1	2.1 ± 0.2
18:0	5.4	5.7 ± 1.9	4.5 ± 1.8	3.5 ± 0.6	4.4 ± 0.5
18:1 (Δ^9)	5.2	11.0 ± 7.7	6.5 ± 1.9	4.9 ± 1.2	6.1 ± 1.1
18:2 ($\Delta^{6,11}$)	5.3	6.7 ± 1.4	8.8 ± 1.7	12.7 ± 1.7	10.5 ± 0.8
18:2 ($\Delta^{9,12}$)	9.7	9.5 ± 1.0	9.5 ± 0.9	8.2 ± 0.2	9.6 ± 0.9
18:3 ($\Delta^{6,9,12}$)	30.7	35.7 ± 7.8	38.5 ± 10.4	50.7 ± 0.6	36.0 ± 2.9
Unsaturated fatty acid	63.6	72.1 ± 5.8	74.1 ± 5.8	85.2 ± 4.2	74.0 ± 1.9
Saturated fatty acid	23.4	16.7 ± 4.8	13.9 ± 7.6	6.2 ± 1.1	13.3 ± 1.5
U/S	2.7	4.7 ± 1.9	7.9 ± 7.0	14.0 ± 3.2	5.6 ± 0.7
Unsaturation index	140	160 ± 22	170 ± 32	206 ± 3	166 ± 7
16:1/16:0	1.1	1.0 ± 0.5	1.5 ± 0.8	2.3 ± 0.1	1.9 ± 1.1
Phosphatidylcholine					
12:0	0.4	0.2 ± 0.1	0.3 ± 0.3	—	0.3 ± 0.3
14:0	8.4	4.7 ± 1.2	4.4 ± 0.7	3.6 ± 0.3	6.0 ± 2.7
15:0 (iso)	3.3	2.1 ± 0.1	2.3 ± 0.1	1.6 ± 0.0	1.3 ± 0.4
15:0	1.8	1.0 ± 0.2	1.0 ± 0.3	1.1 ± 0.1	0.8 ± 0.2
16:0 (iso)	0.5	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.0	0.4 ± 0.1
16:0	15.1	8.8 ± 0.1	6.4 ± 1.4	5.2 ± 0.1	9.4 ± 2.2
16:1	17.2	15.5 ± 2.0	15.5 ± 1.0	11.5 ± 0.1	14.4 ± 1.6
16:2 + 17:0	9.0	10.1 ± 0.1	11.4 ± 1.0	11.8 ± 0.9	8.9 ± 0.6
unknown	2.1	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.2	2.5 ± 0.3
18:0	6.4	5.9 ± 0.9	5.8 ± 0.9	6.5 ± 1.0	7.8 ± 0.8
18:1 (Δ^9)	5.7	5.0 ± 0.1	4.5 ± 1.0	3.6 ± 1.2	6.2 ± 1.7
18:2 ($\Delta^{6,11}$)	2.9	4.7 ± 0.1	5.2 ± 0.1	6.4 ± 0.1	4.7 ± 1.0
18:2 ($\Delta^{9,12}$)	9.0	9.9 ± 1.6	8.9 ± 1.3	6.4 ± 0.4	8.8 ± 1.1
18:3 ($\Delta^{6,9,12}$)	17.7	30.0 ± 3.0	32.2 ± 3.5	40.4 ± 0.6	28.4 ± 7.4
Unsaturated fatty acid	52.5	65.0 ± 2.9	66.2 ± 3.3	68.1 ± 0.9	62.6 ± 6.6
Saturated fatty acid	29.5	17.0 ± 1.9	14.7 ± 2.7	12.0 ± 1.1	18.1 ± 5.7
U/S	1.8	3.9 ± 0.6	4.6 ± 1.0	5.7 ± 0.4	3.7 ± 1.3
Unsaturation index	100	139 ± 11	145 ± 12	162 ± 0	133 ± 23
16:1/16:0	1.1	1.8 ± 0.2	2.5 ± 0.5	2.3 ± 0.4	1.6 ± 0.2

Table I (continued)

Phospholipid	Time after the temperature shift (h)				
	0	2	4	10	24
Phosphatidylethanolamine					
12:0	2.3	0.5 ± 0.5	0.5 ± 0.3	0.6 ± 0.2	1.3 ± 0.3
14:0	14.6	6.2 ± 4.0	8.3 ± 0.4	7.5 ± 0.6	9.3 ± 0.4
15:0 (iso)	5.1	2.9 ± 0.9	3.9 ± 0.5	3.5 ± 0.5	2.4 ± 0.2
15:0	2.2	1.3 ± 0.3	2.6 ± 1.7	1.8 ± 0.4	1.3 ± 0.3
16:0 (iso)	0.6	0.4 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
16:0	15.5	11.7 ± 1.9	8.6 ± 1.2	6.7 ± 0.1	9.5 ± 0.5
16:1	21.7	21.2 ± 1.8	22.5 ± 1.7	20.3 ± 1.6	19.2 ± 1.0
16:2 + 17:0	7.0	8.1 ± 0.3	9.1 ± 1.1	10.5 ± 1.0	8.3 ± 0.5
unknown	2.5	2.5 ± 0.5	3.0 ± 0.7	3.3 ± 0.6	4.2 ± 0.6
18:0	4.0	4.0 ± 0.4	3.6 ± 0.8	4.3 ± 0.6	5.1 ± 0.4
18:1 (Δ^9)	4.8	7.3 ± 1.4	6.2 ± 0.9	7.2 ± 2.3	7.3 ± 1.5
18:2 ($\Delta^{6,11}$)	0.9	1.4 ± 0.3	1.6 ± 0.6	1.7 ± 0.1	2.1 ± 0.2
18:2 ($\Delta^{9,12}$)	8.2	12.5 ± 2.8	10.1 ± 0.1	10.3 ± 1.8	10.6 ± 0.1
18:3 ($\Delta^{6,9,12}$)	9.1	19.6 ± 2.8	17.7 ± 1.2	21.5 ± 1.8	19.2 ± 1.2
Unsaturated fatty acid	44.7	62.1 ± 4.7	53.3 ± 2.2	60.9 ± 4.3	57.4 ± 1.4
Saturated fatty acid	40.3	22.8 ± 3.9	24.5 ± 2.7	20.5 ± 1.7	24.2 ± 1.1
U/S	1.1	2.8 ± 0.7	2.4 ± 0.4	3.0 ± 0.5	2.4 ± 0.2
Unsaturation index	72	115 ± 13	106 ± 4	116 ± 10	107 ± 4
16:1/16:0	1.4	1.9 ± 0.5	2.7 ± 0.5	3.0 ± 0.3	2.0 ± 0.2

unsaturation index, which is highly dependent upon the content of γ-linolenic acid, exhibits a maximum at 10 h in the three phospholipids.

Discussion

When *Tetrahymena* cells are exposed to a new thermal environment, they alter the composition of membrane lipids. In the present experiment, *Tetrahymena* cells quenched to 15 from 39°C alter the fatty acid composition and the lipid fluidity with lapse of time. Since decrease in growth temperature renders the membrane less fluid, cells attempt to recover the proper membrane fluidity by changing the lipid composition [8,10,12]. When the phospholipids extracted from cells taken at different intervals after the temperature shift were examined by electron spin resonance, the fluidity of 2-aminoethylphosphonolipid was found to increase for the first 10 h after the temperature shift. Then further incubation up to 24 h at 15°C resulted in decrease of the fluidity to a value between 2 and 10 h. On the other hand, the content of γ-linolenic acid increased from 35.7% at 2 h to 50.7% at 10 h, and decreased then to 36.0% at 24 h after the temperature shift. The result of lipid fluidity is well compatible with that of fatty acid composition, which implies that the fluidity depends largely upon the content of γ-linolenic acid in the case of 2-aminoethylphosphonolipid. There is some fluidity difference between at 2 and 24 h in spite of the very close content of γ-linolenic acid, which may be explained by the presence of other fatty acids, for example, palmitoleic acid.

On the other hand, the fluidities of phosphatidylcholine and phosphatidylethanolamine were found to increase as the incubation time increased. But these phospholipids showed the highest content of γ-linolenic acid at 10 h.

Thus it is not possible to explain the fluidity changes solely by the content of γ-linolenic acid. The proportional content of γ-linolenic acid and its change during adaptation were small as compared with those of 2-aminoethylphosphonolipid. The contribution of other fatty acids to the fluidity modulation should be taken into consideration. Further decrease in phosphatidylcholine fluidity, observed at more than 10 h after the shift, might be attributed to an increase in $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ content. In the case of phosphatidylethanolamine, however, the decreased fluidity at 24 h could not be explained by the content of $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$, because these contents were almost constant during the intervals examined. Increase in content of short-chain fatty acids, $C_{12:0}$ and $C_{14:0}$, might play an important role for decreasing the fluidity of phosphatidylethanolamine. It should be noted that the positional distribution of fatty acids is presumed to participate in the phospholipid dynamic structure, and that it may play an important role for the activity of membrane enzymes [21]. Indeed, we have examined the positional distribution of fatty acids in phospahtidylethanolamine from *T. pyriformis* cells at different growth temperatures and found that the distribution is altered by the growth temperature [22].

The adaptation process inferred by the movement of membrane particles was almost completed at 11 h after the temperature shift [13]. No sign of further increase in γ-linolenic acid content might reflect the termination of adaptation mechanism which has been operating, and the decrease of 2-aminoethylphosphonolipid might compensate for the over-adaptation of fluidity caused by the other two phospholipids. Evidence was thus presented that an unusual high content of γ-linolenic acid in 2-aminoethylphosphonolipid probably is important for regulating the temperature acclimation mechanism in *Tetrahymena*.

References

1 Sinensky, M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 522—525
2 Rottem, S., Markowitz, O. and Razin, S. (1978) Eur. J. Biochem. 85, 445—450
3 McElhaney, R.N. and Souza, K.A. (1976) Biochim. Biophys. Acta 443, 348—359
4 Cullen, J., Phillips, M.C. and Shipley, G.G. (1971) Biochem. J. 125, 733—742
5 Friedman, K.J. (1977) J. Membrane Biol. 32, 33—47
6 Ferguson, K.A., Glaser, M., Bayer, W.H. and Vagelos, P.R. (1975) Biochemistry 14, 146—151
7 Sandermann, H. Jr. (1978) Biochim. Biophys. Acta 515, 209—237
8 Thompson, G.A. Jr. and Nozawa, Y. (1977) Biochim. Biophys. Acta 472, 55—92
9 Wunderlich, F., Speth, B., Batz, W. and Kleinig, H. (1973) Biochim. Biophys. Acta 298, 39—49
10 Nozawa, Y., Iida, H., Fukushima, H., Ohki, K. and Ohnishi, S. (1974) Biochim. Biophys. Acta 367, 134—147
11 Fukushima, H., Nagao, S., Okano, Y. and Nozawa, Y. (1977) Biochim. Biophys. Acta 488, 442—453
12 Nozawa, Y. and Kasai, R. (1978) Biochim. Biophys. Acta 529, 54—66
13 Martin, C.E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L. and Thompson, G.A. Jr. (1976) Biochemistry 15, 5218—5227
14 Martin, C.E. and Thompson, G.A. Jr. (1978) Biochemistry 17, 3581—3586
15 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911—917
16 Fukushima, H., Martin, C.E., Iida, H., Kitajima, Y., Thompson, G.A. Jr. and Nozawa, Y. (1976) Biochim. Biophys. Acta 431, 165—179
17 Waggoner, A.S., Kingzett, T.J., Rottschaefer, S., Griffith, O.H. and Keith, A.D. (1969) Chem. Phys. Lipids 3, 245—251
18 Berger, H., Jones, P. and Hanahan, D.J. (1972) Biochim. Biophys. Acta 260, 617—629
19 Junger, E. and Reinauer, H. (1969) Biochim. Biophys. Acta 183, 304—308
20 Tinker, D.O. and Pinteric, L. (1971) Biochemistry 10, 860—865
21 Baldassare, J.J., Benckle, G.M., Hoffman, M. and Silbert, D.F. (1977) J. Biol. Chem. 252, 8797—8803
22 Watanabe, T., Fukushima, H. and Nozawa, Y. (1979) Biochim. Biophys. Acta. 575, 365—374