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Microsomal Phospholipid Molecular Species Alterations during Low Temperature Acclimation in *Dunaliella*[1]

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ABSTRACT

A detailed analysis of the low temperature-induced alterations of *Dunaliella salina* (UTEX 1644) microsomal membrane lipids was carried out. Microsomal membranes were isolated from cells grown at 30°C, from cells shifted to 12°C for 12 hours, and from cells acclimated to 12°C. Fatty acid analyses of the major lipid classes demonstrated significant changes in the fatty acid composition of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) but not phosphatidylcholine (PC) during the initial 12 hours at low temperature. These changes did not entail enhanced desaturation of linoleic acid. Subsequent to 12 hours, the proportions of linolenic acid increased in all phospholipids.

Molecular species analyses of the phospholipids demonstrated that the most immediate changes following a shift to low temperature were limited to several molecular species of PE and PG. The changes observed in PE included a decrease in C_{30} species and concomitant increases in C_{34} and C_{36} species. Compositional changes associated with PG entailed the emergence of a new molecular species (18:1/18:1) not found at 30°C. The retailoring of molecular species resulted in an increase in the number of species having two unsaturated acyl chains and did not reflect a simple enhancement of desaturase activity as suggested by the fatty acid analysis. We conclude that the initial alterations in response to low temperature stress involve discrete changes in certain molecular species. These and further alterations of molecular species following acclimation to low temperature would appear to augment increases in acyl chain desaturation as a means of modifying membrane properties in response to low temperature stress.

In response to chilling, many poikilothermic organisms exhibit alterations in membrane lipid composition, enabling them to overcome the deleterious effects of low temperature on membrane physical properties and membrane-related functions. The most commonly reported changes consist of an increase in unsaturation of the acyl chains associated with membrane lipids (1, 3). Other mechanisms of lipid retailoring may also occur in response to chilling temperatures (6, 15).

Although plant microsomal enzymes catalyze many key metabolic reactions, few studies have focused on the lipid compositional alterations of these membranes in response to chilling. However, the response of the microsomal membranes of the protozoan *Tetrahymena pyriformis* in response to low temperature has been studied in detail (5, 6). A correlation was found between changes in physical properties and alterations in the pattern of phospholipid molecular species, *i.e.* the combinations of fatty acids occurring together in the same phospholipid molecule (6). Such observations are of special interest in light of recent reports which suggest that physical properties of plant membranes may be strongly influenced by structural parameters of the lipids other than acyl chain composition *per se* (11, 14).

In this communication, we describe the changes in microsomal membrane acyl chain composition of each major polar lipid of the green alga *Dunaliella salina* and demonstrate that the molecular species distribution within certain phospholipid classes is rapidly modified by low temperature stress. This analytical approach permits the recognition of structural and metabolic relationships not revealed by usual lipid analytical techniques. Similar analyses have also been carried out on chloroplast phospholipids and are presented in the accompanying paper.

MATERIALS AND METHODS

Culture Conditions. Axenic cultures of *Dunaliella salina* (UTEX 1644) were grown in synthetic medium under conditions previously described (7). Cultures were grown isothermally at 30°C or chilled slowly (over 2.5 h) to 12°C and maintained at 12°C for the designated time. Cells held at 12°C for longer than 100 h resumed logarithmic growth at low temperature and were considered as being 12°C-acclimated cells. It has been found that these cells have essentially the same lipid composition as cells grown at 12°C for much longer periods.

Membrane Isolation. Cells were harvested in the middle to late logarithmic growth phase and fractionated as previously described (7) except that the cell suspension was allowed to equilibrate in the Parr bomb for 15 rather than 10 min. The 20,000*g* supernatant was centrifuged at 105,000*g* for 1 h to obtain the microsomal fraction.

Lipid Analyses. Lipids were extracted using the procedure of Bligh and Dyer (2). Total phospholipids (including DGTH,[3] a non-phosphorus-containing polar lipid [9]) were obtained by silicic acid column chromatography (9). The individual lipid classes (PC, PG, PE, and DGTH) were isolated by TLC on silica gel H using chloroform:acetic acid:methanol:water (70:25:5:2.2, v/v/v/v) as the solvent system. After flushing the plate with N_2 for 2 to 5 min to remove the solvents, the lipid bands were detected by a brief exposure to iodine vapors. For molecular species and/or methyl ester analyses (8), the bands were immediately scraped off the plate, and the lipids were eluted from the silica gel using chloroform:methanol:water (3:5:1, v/v/v), then dried under N_2. Alternatively, methyl esters were formed in the presence of silica gel by scraping the lipid bands directly into

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[3] Abbreviations: DGTH, diacylglyceryltrimethylhomoserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; TMS, trimethylsilyl.

BF_3/methanol (Sigma) and heated at 100°C in sealed ampoules for 10 min. In both cases, oxidation of polyunsaturated fatty acids was negligible as determined by fatty acid analyses before and after treatment.

Phospholipids were converted to diacylglycerols by incubating the lipid with phospholipase C at 37°C for 1.5 to 2 h in a reaction mixture made by adding 30 to 35 μl enzyme suspension (phospholipase C grade II from *Bacillus cereus*; Boehringer Mannheim) and 0.25 ml buffer (10 mM phosphate [pH 7.4], 0.25 mM $CaCl_2$, 100 μM $ZnCl_2$) to lipid resuspended in 1 ml peroxide free ethyl ether. The diacylglycerols were extracted by removal of the ether phase and subsequent re-extraction of the aqueous phase with ethyl ether:petroleum ether (1:1,v/v). Conversion of phospholipid to diacylglycerol was 95 to 98% complete as determined using lipids prelabeled biosynthetically with [^{14}C]oleic acid. Phospholipase C was ineffective in converting DGTH to a diacylglycerol; thus, a molecular species analysis was not feasible.

Diacylglycerols were converted to trimethylsilyl derivatives by treatment with 0.25 to 0.5 ml Sylon HTP (Supelco; pyridine:hexamethyldisilazane:trimethylchlorosilane, 9:3:1 [v/v/v]) for 30 to 60 min at 35°C. The mixture was then dried under N_2 and the derivatives were extracted in petroleum ether (10).

Gas chromatography was performed on a Varian 3700 GC using a 10 m × 0.25 mm i.d. open tubular glass column coated with SP2330 (Supelco). The injector and detector components were deactivated prior to initial installation of the column. For fatty acid methyl ester analysis, the column temperature was maintained at 170°C and N_2 head pressure was set at 0.4 kg cm^{-2}. Injector and detector temperatures were both held at 230°C. Split injection (split ratio set at 40:1 to 100:1) was used. For analyses of the TMS ethers of the diacylglycerols, the column temperature was programmed from an initial temperature of 200°C (held for 5 min) to 250°C at 10°C min^{-1} and the N_2 head pressure was maintained at 0.5 kg cm^{-2}. Injector and detector temperatures were 270° and 300°C, respectively. A split ratio of 10:1 to 20:1 was used.

A variety of procedures was used to identify molecular species, including co-chromatography with TMS derivatives of standards, chromatography of derivatives following argentation TLC of the constituent diacylglycerols (on 10% $AgNO_3$ impregnated silica gel H developed in chloroform:ethanol (95:5, v/v) (16)), comparison of fatty acid composition and molecular species composition of certain phospholipids, and GC-MS analysis of the *t*-butyldimethylsilyl derivative (6). Although these were satisfactory for the identification of major peaks, the limited resolution of the GC-MS and the inability to recover minor components following argentation TLC led us to identify some of the molecular species only tentatively or include them in with an identified peak having the (presumed) same carbon number and total overall number of double bonds.

Gas chromatography of the molecular species reported here was in accord with the technique published by Myher and Kuksis (10). Differences in operating conditions or carrier gas (N_2 *versus* H_2) may be responsible for the differences in chromatographic properties reported here, including a somewhat diminished recovery of long chain polyunsaturated species in favor of very short chain species having short retention times. This is most evident for microsomal PE, which contains a high proportion of a short chain component (14:2). The relative percentage of 14:2 calculated from molecular species data was approximately 28% whereas 14:2 content determined by fatty acid analysis was found to be 21%. In this case, the molecular species span a range of six carbons (C_{30} to C_{36}) and six double bonds. The slight discrimination against the higher mol wt derivatives presented no problem in making the relative comparisons described below. A comparison of the calculated fatty acid composition from molecular species data *versus* methyl ester analyses was in good agreement (within 2–4%) for the other phospholipids.

To further characterize the molecular species composition, individual phospholipids were treated with phospholipase A_2 as previously described (17) and the positional specificity of most of the acyl chains was determined.

RESULTS

Earlier work employing *Dunaliella* as a model system to study plant membrane lipid dynamics demonstrated that within 12 h following a shift to low growth temperature, small but significant alterations in the acyl chain composition of the microsomal phospholipids occurred (7). Equivalent changes in chloroplast phospholipids were not detected until 60 h after chilling. In order to more fully realize the extent and the pattern of the initial temperature related compositional changes occurring in the microsomal compartment, fatty acid analyses of the major polar lipids were undertaken. For this purpose, a comparison was made of 30°C-grown cells, cells shifted to 12°C for 12 h, and cells acclimated to 12°C by exposure to that temperature for 100 h or more.

The fatty acid compositions of the total phospholipid fraction (including the unique non-phosphorus-containing polar lipid DGTH; see Ref. 9 for structure) of the microsomes (data not shown) confirmed the same trends reported earlier (7). The fatty acid compositions of the individual phospholipid classes are shown in Table I. It is noteworthy that microsomal PG contained primarily $18:2^{\Delta 6,9}$, whereas chloroplast PG contained $18:2^{\Delta 9,12}$ (see accompanying paper). The positional distribution was also different (see below).

The trends in acyl chain alteration during acclimation were towards an increase in unsaturation, most noticeably, increases in both positional isomers of 18:3 at the expense of 14:2, 16:0, and 18:2. During the initial 12-h period at low temperature, however, the microsomal phospholipids experienced a number of significant changes in acyl chain composition other than a general increase in 18:3 content. Most dramatic were the alterations in PE involving a sharp drop in 14:2 and 16:0, accompanied by a rise in 18:1 and 18:2. These early changes in PE quickly established an acyl chain pattern that remained basically unaltered during the rest of the acclimation period.

Rapid alterations in the PG acyl chain composition were also evident, but were not as large as those associated with PE. Many of these appeared to be transient in that some of the differences between 30 and 12°C values were less than those between 30°C and 12-h-shifted values (*e.g.* 18:1 and $18:2^{\Delta 6,9}$) (Table I). Palmitic acid decreased within 12 h to the level found in 12°C-acclimated microsomal lipids. The increased 18:3 content observed in PE and PG from 12°C-acclimated samples was established at some time following a 12-h shift.

During a 12-h shift, DGTH displayed a pronounced decrease in the relative proportion of 16:0 and slight increases in the proportions of 18:2 and both isomers of 18:3. Subsequently, there was a continued increase in the relative amounts of 18:3 isomers at the expense of 18:2.

Little or no change in the acyl chain composition of PC occurred during the initial 12 h of chilling. The eventual compositional alterations, including decreases in 16:0 and 18:2 and increases in both 18:3 isomers, occurred during later stages of acclimation.

Information regarding the intramolecular pairing of phospholipid acyl chains was obtained by gas chromatographic analyses of molecular species. This technique allowed a clear resolution of most molecular species based on carbon number and number of double bonds. The relative changes in molecular species composition associated with temperature acclimation are shown in Table II. The most dramatic alteration was the rapid decrease, from 58 to 31%, in 16:0/14:2 associated with PE during a 12-h

Table I. *Effects of Chilling on Phospholipid Fatty Acid Composition of Microsomal Membranes*

Cells were grown at 30°C or acclimated to 12°C, or shifted to 12°C for 12 h. Values are expressed as mean per cent of total fatty acid weight ± SE of three to five separate experiments.

Lipid Class	14:1[a]	14:2	16:0	16:1	18:0	18:1	18:2 (Δ6,9)	18:2 (Δ9,12)	18:3 (Δ6,9,12)	18:3 (Δ9,12,15)	18:4
PC											
30°C	0.7 ± 0.2	0.6 ± 0.2	37.7 ± 0.4	tr[b]	2.2 ± 0.4	10.1 ± 2.6	—[c]	33.7 ± 2.5	4.8 ± 0.7	10.3 ± 0.8	tr
12 h	0.5 ± 0.1	0.5 ± 0.1	36.5 ± 0.1	tr-0.2	1.9 ± 0.3	9.7 ± 1.3	—	36.0 ± 0.4	6.2 ± 0.4	8.8 ± 0.6	tr
12°C	0.7 ± 0.1	1.0 ± 0.1	28.8 ± 0.5	tr-0.3	0.8 ± 0.2	12.7 ± 0.7	—	28.3 ± 0.5	10.9 ± 0.4	16.3 ± 1.1	tr-0.4
PG											
30°C	0.4 ± 0.1	0.9 ± 0.2	54.5 ± 2.0	4.5 ± 0.2	0.6 ± 0.1	7.3 ± 0.1	26.9 ± 2.8	3.0 ± 0.1	0.6 ± 0.1	1.8 ± 0.7	—
12 h	1.1 ± 0.4	2.2 ± 0.8	49.8 ± 0.6	7.0 ± 0.4	tr-0.4	12.6 ± 0.8	20.1 ± 0.3	4.6 ± 0.2	0.5 ± 0.1	2.4 ± 0.5	—
12°C	1.4 ± 0.2	4.3 ± 0.2	48.4 ± 2.2	5.2 ± 0.4	tr-0.7	8.2 ± 0.2	25.3 ± 1.8	3.0 ± 0.1	1.0 ± 0.1	3.3 ± 0.3	—
PE											
30°C	3.4 ± 0.5	21.2 ± 2.1	51.4 ± 0.3	—	tr-0.4	13.4 ± 0.4	1.0 ± 0.1	5.4 ± 0.9	2.5 ± 0.2	1.8 ± 0.3	—
12 h	2.8 ± 0.8	9.6 ± 0.8	37.8 ± 1.5	—	0.9 ± 0.3	28.8 ± 1.0	1.2 ± 0.1	12.2 ± 0.9	3.7 ± 0.5	2.9 ± 0.3	—
12°C	2.5 ± 0.4	9.6 ± 0.4	35.1 ± 1.0	—	0.4 ± 0.1	27.7 ± 0.5	2.3 ± 0.4	10.7 ± 0.5	6.9 ± 0.2	5.0 ± 1.3	—
DGTH											
30°C	tr-0.6	1.3 ± 0.1	42.9 ± 0.6	tr-0.9	0.4 ± 0.1	3.7 ± 0.2	—	25.8 ± 1.1	10.7 ± 0.1	13.3 ± 1.2	0.4 ± 0.1
12 h	tr	0.6 ± 0.1	37.4 ± 1.1	0.6 ± 0.1	0.9 ± 0.2	3.8 ± 0.1	—	27.6 ± 0.4	12.2 ± 0.2	17.2 ± 0.4	0.5 ± 0.1
12°C	tr	tr-0.3	37.1 ± 0.4	tr	0.5 ± 0.1	6.1 ± 0.5	—	14.5 ± 0.2	15.8 ± 0.4	22.3 ± 0.4	1.9 ± 0.1

[a] The number preceding the colon represents the number of carbon atoms in the fatty acid and the number following the colon indicates the number of double bonds present.

[b] tr, trace.

[c] —, not detected.

Table II. *Effects of Chilling on Phospholipid Molecular Species Composition of Microsomal Membranes*

Cells were grown at 30°C or acclimated to 12°C or shifted to 12°C for 12 h. Values are expressed as mean per cent of total derivative weight ± SE of three to five separate experiments. Underlined values differ markedly from the 30°C value.

Component Molecular Species	PC			PG			PE		
	30°C	30→12°C 12 h	12°C	30°C	30→12°C 12 h	12°C	30°C	30→12°C 12 h	12°C
16:0/14:1[a]	1.6 ± 0.4	2.0 ± 0.3	2.2 ± 0.2	0.8 ± 0.2	1.5 ± 0.8	1.0 ± 0.2	9.6 ± 0.2	10.0 ± 1.3	5.4 ± 0.5
16:0/14:2	2.5 ± 0.3	2.9 ± 0.5	4.4 ± .05	3.3 ± 0.6	4.4 ± 2.0	3.9 ± 0.5	58.7 ± 3.4	31.3 ± 1.9	28.2 ± 1.8
NI[b]	0.4 ± 0.1	0.3	0.5 ± 0.1	tr-0.7	tr-0.5	0.5 ± 0.1	4.0 ± 0.1	2.2 ± 0.3	3.7 ± 0.3
16:0/16:0	—[c]	—	—	4.5 ± 0.3	7.2 ± 0.7	3.3 ± 0.6	—	—	—
18:0/14:1	—	—	—	—	—	—	tr-0.6	2.0 ± 0.2	1.2 ± 0.1
18:0/14:2, 18:1/14:1	0.8 ± 0.2	0.7 ± 0.1	1.0 ± 0.1	—	—	—	1.1 ± 0.2	1.5 ± 0.3	2.8 ± 0.2
16:0/18:1	2.3 ± 0.5	1.1 ± 0.1	2.2 ± 0.5	—	—	—	6.1 ± 0.6	8.8 ± 0.4	6.8 ± 0.2
18:1/16:1	—	—	—	[82.7 ± 1.6[d]	66.9 ± 2.3	70.1 ± 0.8	—	—	—
16:0/18:2$^{\Delta 6,9}$	—	—	—				—	—	—
16:0/18:2$^{\Delta 9,12}$	51.5 ± 1.6	50.6 ± 0.9	38.0 ± 0.3				5.9 ± 0.1	12.0 ± 1.0	12.4 ± 2.5
18:2$^{\Delta 9,12}$/16:1	—	—	—	3.9 ± 0.6	5.7 ± 0.9	5.8 ± 0.9	—	—	—
16:0/18:3$^{\Delta 9,12,15}$	12.6 ± 0.8	11.6 ± 0.1	14.5 ± 0.6	tr-0.6	0.9 ± 0.1	2.1 ± 0.1	0.6 ± 0.1	tr-0.8	1.0 ± 0.1
18:3$^{\Delta 9,12,15}$/16:1	—	—	—	2.7 ± 0.9	5.5 ± 0.5	8.8 ± 0.3	—	—	—
NI	—	—	—	—	—	—	—	—	—
18:1/18:1, 18:0/18:2	1.1 ± 0.3	0.8 ± 0.2	1.3 ± 0.3	—	5.5 ± 0.2	5.2 ± 0.4	3.7 ± 0.1	14.4 ± 1.4	13.3 ± 2.2
18:1/18:2$^{\Delta 9,12}$, 18:0/18:3	3.6 ± 0.8	4.5 ± 0.5	10.0 ± 0.4	—	—	—	4.2 ± 0.2	11.3 ± 1.3	13.3 ± 1.0
18:2$^{\Delta 9,12}$/18:2$^{\Delta 9,12}$	11.1 ± 0.4	13.0 ± 0.7	9.1 ± 0.5	—	—	—	[3.6 ± 0.4	4.6 ± 0.8	6.6 ± 0.8
18:1/18:3$^{\Delta 9,12,15}$, 18:1/18:3$^{\Delta 6,9,12}$	2.8 ± 0.3	3.0 ± 0.2	5.8 ± 0.6	—	—	—			—
18:2/18:3$^{\Delta 6,9,12}$	5.4 ± 0.6	4.8 ± 0.4	6.1 ± 0.2	—	—	—	0.8 ± 0.1	tr-0.9	1.5 ± 0.3
18:2/18:3$^{\Delta 9,12,15}$	0.8 ± 0.2	0.5 ± 0.1	1.6 ± 0.3	—	—	—	—	—	—
18:3$^{\Delta 9,12,15}$/18:3	tr-0.3	0.5 ± 0.1	1.3 ± 0.1	—	—	—	—	—	—

[a] The acyl chains separated by a slash represent the components in the *sn*-1 and *sn*-2 positions, respectively, of the molecular species.

[b] NI, not identified.

[c] —, not detected.

[d] Brackets signify that the included molecular species were not fully resolved. The numerical value is placed adjacent to the identity of the most abundant species.

exposure to low growth temperature. Compensatory increases of 6 to 10% in the proportions of 16:0/18:2, 18:1/18:1, and 18:1/18:2 were observed. Changes occurring after a 12-h shift to low temperature included a decrease in 16:0/14:1 and increases in the 18:3-containing peaks, in particular 18:1/18:3 and 18:2/18:3.

Microsomal PG was composed mainly of 16:0/18:2. This major peak decreased from 83 to 62% of the total microsomal PG during the first 12 h at 12°C. The appearance of 18:1/18:1, a 'new' molecular species, accompanied the other rapid alterations associated with temperature acclimation. It is of interest to note that microsomal PG was the only lipid analyzed which contained a totally saturated species, 16:0/16:0.

In contrast to the other two phospholipids, PC molecular species demonstrated little or no compositional alterations during the initial 12 h at low temperature. We will show in the accompanying paper that the molecular species of chloroplast PC are strikingly similar to those of microsomal PC whereas the molecular species distribution of the respective PE and PG classes of the two fractions are quantitatively quite different. The most apparent difference in the PC molecular species between 30°C-grown and 12°C-acclimated samples was the 13.5% decrease in 16:0/18:2. There was only a slight corresponding rise in 16:0/18:3. Instead, the bulk of the change involved increases in the 36-carbon molecular species, including 18:1/18:2, 18:1/18:3, 18:2/18:3, and 18:3/18:3.

DISCUSSION

The aim of this study was to examine in detail the timing and the extent of lipid compositional alterations which occur in the microsomal membranes during low temperature acclimation. In spite of the multiple involvements of plant microsomal membranes in lipid metabolism, the effects of chilling temperatures on their lipid composition and functioning have been examined only sparingly (1, 5, 7).

Individual lipid classes of microsomes respond to chilling by altering their acyl chain composition in different ways and at different rates (Table I). Molecular species analysis is even more informative, in that it reveals not only the sharp differences in overall acyl chain composition but also the dramatic fluctuations in constituent pairing (Table II). Thus, in response to a shift in growth temperature, a given molecular species containing a particular acyl chain may increase or decrease in amount while another species containing the same acyl chain many react quite differently. A full accounting of these changes is essential if we hope to gain a complete understanding of the various metabolic activities which regulate or influence lipid composition and membrane function.

In this study, the rapid retailoring of certain phospholipid molecular species overshadowed the general increase in fatty acid unsaturation usually associated with low temperature acclimation. This retailoring resulted in an increase in the proportion of molecular species having two unsaturated acyl chains at the expense of species which have one saturated acyl chain. Rapid changes were also reported to occur in *Tetrahymena* microsomal membrane lipids following a shift in growth temperature for 1 h (6). During this short time period, 14:0 and 16:0 decreased somewhat, whereas 18-carbon fatty acids increased. Molecular species analyses using GC-MS indicated that C_{33} and C_{34} species decreased sharply and C_{30}, C_{31}, and C_{36} species increased. Thus, in both organisms, the most rapid changes involved acyl group rearrangement. Increases in 18:3 content occurred following longer periods at low growth temperatures. Detailed analyses of lipids from a number of different organisms will be necessary to decide if this type of change is a common response to stress. To date, no other eukaryotic plant cells have been so analyzed; however, similar findings were derived from studies of the prokaryotic alga *Anabaena variabilis* (15). After chilling, the membranes became temporarily enriched in molecular species containing two unsaturated acyl chains. This pattern of change was postulated to represent an 'emergency response' needed until increases in 18:3 content could be achieved.

It is interesting that certain phospholipids altered their constituent acyl chain (and molecular species) compositions more rapidly and to a greater extent than did others. This was also reported to occur in *Tetrahymena* microsomal phospholipids (6) and *Nerium oleander* chloroplast polar lipids (14). In this last example, however, only acyl chain compositions were analyzed.

The mechanism by which *Dunaliella* rapidly alters its microsomal phospholipid molecular species composition is not clear, but several possibilities exist. These include alterations in the synthesis, transport, and/or deacylation/reacylation of fatty acids required for phospholipid assembly. Analysis of the acyl chain composition of PE, the most responsive lipid, immediately after the culture attained a temperature of 12°C (2.5 to 3 h after placing the 30°C culture in the 12°C incubator) indicated that little, if any, alteration occurred during the cooling process itself. Thus, it is clear that whatever the mechanism, it is operable at 12°C and may be effectively studied under isothermal conditions.

Lipid changes of the type shown here can have profound effects on membrane physical properties and function. Rapid changes in *Tetrahymena* microsomal membrane physical properties following a shift in growth temperature (5) were found to correlate with changes in phospholipid molecular species rather than with acyl chain composition (6). It has been demonstrated using pure synthetic phospholipids and simple mixtures of such lipids that physical properties of the lipids in bilayers cannot be predicted on the basis of the overall fatty acid composition alone. The intramolecular positioning of the fatty acids is itself an important factor in determining the fluidity of lipid assemblages (12, 13).

It was found in this study that in response to low temperature PC 16:0/18:2 decreased, but 16:0/18:3 did not increase significantly. Instead, other 18:2- and 18:3-containing species increased. It has been demonstrated that, for PC, the mean areas occupied by 16:0/18:2 and 16:0/18:3 are essentially the same (4). Liposomes composed of PC having either of these two molecular species displayed similar permeability properties. The same measurements made using PC containing two unsaturated chains (18:2/18:2 or 18:3/18:3) demonstrated that their physical properties differed dramatically from the saturated chain-containing species. This suggests that certain species which have a more fluidizing effect, such as the C_{36} species, are maintained or increased during acclimation to low temperature whereas 'less fluidizing' species (16:0/18:2, 16:0/18:3) are diminished. Thus, it appears that the molecular species changes of PC associated with chilling in *Dunaliella* are the ones that would most enhance fluidity and thus augment the effects of increased acyl chain unsaturation. A similar pattern of change was observed in PE and PG, the two other major phospholipid classes. Additional data from studies of chloroplast lipids are described in the accompanying paper. Indeed, acylating and deacylating enzymes responsible for establishing the specific molecular species may play a key role in acclimation to environmental stress. Unfortunately, any such general hypothesis must be considered speculative until more information about the physical properties of other unsaturated phospholipids (such as PE) is available.

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