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

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ABSTRACT

The alterations in chloroplast phospholipid acyl chain composition and phospholipid molecular species composition of *Dunaliella salina* (UTEX 1644) were monitored during acclimation to low temperature. Chlorophyll fluorescence yield, an indicator of chloroplast membrane stability, was used as a physical means of following the acclimation process.

Minor alterations in phospholipid acyl chain composition were evident within 36 hours of shifting the cells from 30 to 12°C. Between 36 and 60 hours, pronounced changes in the acyl chain composition of phosphatidylglycerol (PG) were observed. Changes in the acyl chain composition of phosphatidylcholine (PC) did not occur until sometime after 60 hours.

Alterations in the phospholipid molecular species during acclimation were also examined. The pattern of change observed in PC molecular species, namely a decrease in species having one saturated chain (16:0) paired with a C_{18} acyl chain and a concomitant increase in species having two unsaturated C_{18} acyl chains, suggests that molecular species changes augment fatty acid compositional changes as a mean of adapting to low temperature. The molecular species of PG were found to change abruptly between 36 and 60 hours following a shift to low temperature. During this time, a dramatic alteration in the threshold temperature of thermal denaturation of the photosynthetic apparatus, as measured by chlorophyll fluorescence, also occurred. Lipid compositional changes other than those associated with PG were negligible during this time. This strongly suggests that a correlation exists between the molecular species composition of PG and the thermal stability of the photosynthetic membrane.

The mechanisms involved in the acclimation of plants to low temperature are not well understood (2, 21). Changes in cell membranes and their constituent lipids have been observed in response to chilling. Earlier work from our laboratory demonstrated that the phospholipid acyl chains of *Dunaliella* chloroplast membranes become more unsaturated following a drop in growth temperature (9). Similar changes in the lipid composition of photosynthetic membranes in response to chilling have been observed to occur in a variety of plants (21) and these are thought to be responsible for increased membrane fluidity in these tissues.

On the other hand, the fluidization of membrane lipids of *Nerium oleander* (17) and species of *Passiflora* (13) were not well correlated with increased acyl chain unsaturation. Consequently, it would seem that some membrane lipid structural parameter other than fatty acid composition must play a role in determining the key physical properties which control physiological activities.

An alternative mechanism for bringing about increased membrane fluidity in response to low temperature stress has been detected in *Tetrahymena* (5, 6). Detailed molecular species analyses using coupled GC-MS (6) suggested that rapid changes in the lipid physical properties were due to alterations in the phospholipid molecular species. Physical studies of pure phospholipids and simple phospholipid mixtures have demonstrated that the inter- and intramolecular exchange of acyl chains can markedly alter the physical properties of the lipids (4, 15, 16).

In this communication, alterations in the acyl chain compositions of the individual lipid classes and the phospholipid molecular species compositions of chloroplast membranes during low temperature acclimation are described. Physical changes in the photosynthetic membrane associated with temperature acclimation are reflected in the threshold temperature of enhanced Chl fluorescence. Here, we present data which show that the extent and the time course of lipid changes in the chloroplast fraction differ from the microsomal patterns (see accompanying paper), and that certain molecular species changes reflect closely the changes in Chl fluorescence behavior.

MATERIALS AND METHODS

Culture Conditions. Cultures of *Dunaliella salina* (UTEX 1644) were grown as previously described (9). Cells were grown at 30°C or shifted to 12°C and maintained at that temperature for the designated time. Cells which had resumed logarithmic growth at low temperature (about 100 h following a temperature shift) were used as 12°C-acclimated cells.

Membrane Isolation. The 2000 *g* pellet obtained following cell disruption (9) was used as the starting membrane material. This pellet has already been shown to be highly enriched in chloroplasts with only minor cellular contamination (9).

Lipid Analyses. Lipids were extracted using the procedure of Bligh and Dyer (3). The separation of lipids by silicic acid column chromatography (11) and TLC (accompanying paper) has been previously described. Details of the lipid analyses, including modifications of the procedures of Myher and Kuksis (12) for the analysis of molecular species, identification of molecular species, and determination of acyl chain positional locations (20) are presented in the accompanying paper. Chl content and galactolipid ratios were determined as previously described (9). The technique for molecular analyses of MGDG[3] and DGDG using HPLC has recently been presented (10).

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[3] Abbreviations: DGTH, diacylglyceryltrimethylhomoserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; TMS, trimethylsilyl; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; SL, sulfoquinovosyldiglyceride.

Chlorophyll Fluorescence. Fluorescence yield (F_0) measurements were made using an American Instrument Co. model SPF-125 spectrophotofluorometer with a xenon lamp. The sample (cell suspension) was continuously excited at a wavelength of 340 nm with a 0.1-mm slit width (1.5-nm spectral bandpass) having an intensity of 0.7 to 0.8 ergs cm^{-2} s^{-1}. Fluorescence emission was measured at 670 nm using a slit width of 4 mm (44-nm bandpass). The instrument was set on its most sensitive scale.

Cells at low density (5–15 × 10^4 cells ml^{-1}) were placed in a jacketted cuvette coupled to a temperature-controlled recirculating water bath. The temperature was raised at a rate of about 1°C min^{-1}. Sample temperature was monitored using a thermocouple placed in the sample. Measurements were made by simultaneously noting the temperature and the relative fluorescence. In these experiments, cell death occurred at about 45°C, but the chloroplasts (and the cells) retained their gross structural integrity throughout the entire heating range.

RESULTS

It was previously demonstrated that a variety of compositional changes in the chloroplast membrane lipids of *Dunaliella* are associated with acclimation to low temperature stress. The most conspicuous differences noted in the fully acclimated cells were associated with the phospholipid fatty acids (9); however, only slight changes were detected in the chloroplast phospholipid fatty acids within 60 h after shifting the cultures from 30 to 12°C. More pronounced changes occurred after this time.

To further characterize the changes associated with the chloroplast membrane, fatty acid analyses of the major phospholipids and DGTH, a non-phosphorus-containing polar lipid, were performed. A comparison was made of chloroplast lipids from cells grown at 30°C, cells acclimated to 12°C (by exposure to that temperature for at least 100 h), and cells which were shifted from the high to the low temperature for 12, 36, and 60 h.

The alteration in total phospholipid acyl chain composition during acclimation was similar to that published earlier (9). No change in composition was detected following 12 h at low temperature; however, $18{:}2^{\Delta 9,12}$ decreased from 26.3 to 20.9% and both isomers of 18:3 increased slightly by 36 h (data not shown). More pronounced changes following the same trends were evident in 60-h shifted and 12°C-acclimated samples. Following 60 h, there was also an increase in the proportion of 16:0 and a concomitant decrease in the proportion of 16:1. Based on its association with the *sn*-2 position of PG, this is believed to be $16{:}1^{\Delta trans\text{-}3}$.

The individual lipid classes demonstrated quite different acyl chain compositions and alterations in response to low temperature (Table I). Both isomers of 18:3 associated with DGTH increased with 36 h of shifting and continued to increase during the entire period of acclimation.

The fatty acids of PE were found not to change within the first 12 h. In 12°C-acclimated samples, the proportions of 16:0 and 18:1 were lower and the proportions of both isomers of 18:3 were higher in comparison to 30°C-grown samples.

Phosphatidylglycerol displayed the most complex pattern of change. Small changes in the proportions of 18:1 and 18:2 were observed within 36 h of chilling. Between 36 and 60 h, 18:2 decreased and 18:3 increased almost to their values in 12°C acclimated samples. A small increase in 16:0 and decrease in 16:1 during this time period foreshadowed the further changes in these two fatty acids which occurred after 60 h of chilling.

Phosphatidylcholine showed little change at the time points investigated. In chloroplasts of 12°C-acclimated cells, however, 16:0 and 18:2 decreased whereas 18:1 and both isomers of 18:3 increased.

A gas chromatographic analysis of diglyceride-TMS derivatives revealed that, during low temperature acclimation, the major molecular species of PC, 16:0/18:2, decreased; however, no concomitant rise in 16:0/18:3 was observed. Rather, C_{36} species, such as 18:1/18:2, 18:1/18:3, and 18:2/18:3 increased. The species 18:2/18:2 was also found to decrease somewhat. The temperature-induced changes in these species are strikingly similar to those of microsomal PC (accompanying paper). The timing of these changes was followed by examining the molecular species composition following 12, 36, and 60 h at 12°C. All the major changes were found to occur following 60 h at low temperature (Table II).

In contrast to microsomal PE, which demonstrated dramatic changes during the first 12 h at 12°C, the molecular species composition of PE present in the chloroplast fraction did not change over that time period. The overall changes which did ultimately occur in PE of the chloroplast fractions from chilled cells were also less pronounced than in the microsomal samples. Because these changes were small and PE is a minor component, further time points were not examined.

Interesting changes in chloroplast PG molecular species occurred during acclimation. After an initial 36 h without change, the 18:2/16:1 species of PG decreased from 34 to 26% between 36 and 60 h. After 60 h, it decreased somewhat more, to a final value of 21% in 12°C-acclimated cells. More dramatic was the alteration in the predominant molecular species, 18:3/16:1. After remaining essentially unchanged through 36 h of chilling, the proportion of 18:3/16:1 increased from 48 to 57% between 36 and 60 h. No additional change of any significance was found to occur following 60 h at 12°C.

It is important to learn whether these lipid compositional changes affect the physical properties and function of the acclimating chloroplast. Towards this end, Chl fluorescence measurements were carried out on 30°C-grown cells, 12°C-acclimated cells, and cells shifted to 12°C for 12, 36, 60, and 80 h. The results are shown as relative fluorescence *versus* temperature curves in Figure 1. The figure reveals that with increasing time at low temperature, there was an abrupt shift in the temperature at which enhanced Chl fluorescence occurred. In 30°C-grown cells, Chl fluorescence began to increase upon heating the cells to 66°C. Generally similar patterns were obtained for cells which had previously been shifted from 30 to 12°C for 12 or 36 h. However, in cells shifted from high to low temperature for 60 h, enhanced fluorescence was observed at considerably lower temperatures: 48 to 50°C. Similar curves were obtained for 80-h-shifted cells and 12°C-acclimated cells.

The dramatic alteration in the fluorescence *versus* temperature curves following a shift in growth temperature between 36 and 60 h correlated well with changes in the molecular species of chloroplast PG (Table II). A single analysis of PG molecular species and Chl fluorescence data from cells shifted to 12°C for about 54 h produced values for the two parameters which were between those of 36 and 60-h-shifted cells, providing further evidence of a correlation (data not shown).

The timing and the extent of changes in the molecular species of PC or in the acyl chain compositions of the lipids in Table I did not coincide with the changes in the threshold temperature for Chl fluorescence. To determine if other lipid compositional changes paralleled the changes in Chl fluorescence, we also analyzed Chl content, Chl *a*/*b* ratio, galactolipid acyl chain composition, MGDG and DGDG acyl chain compositions, and MGDG and DGDG molecular species composition (using HPLC [10]). None of these parameters showed any significant change between 36 and 60 h following a shift in growth temperature (data not shown).

Analysis of the galactolipid content indicated that between 36 and 60 h, the proportion of MGDG decreased by 2.5 mol % and that of DGDG increased by 2.5 mol %. This change in galacto-

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

Cells were grown at 30°C or acclimated to 12°C, or shifted to 12°C for 12, 36, or 60 h. Values are expressed as mean per cent of total fatty acid weight ± SE of three to five separate experiments except where noted.

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	tr[b]-0.5	0.8 ± 0.1	34.2 ± 0.4	tr-0.4	1.0 ± 0.2	5.8 ± 0.9	—[c]	36.4 ± 1.5	5.0 ± 0.7	14.5 ± 0.2	—
12 h	0.4 ± 0.1	0.5 ± 0.1	33.1 ± 1.1	tr-1.4	2.1 ± 0.4	7.9 ± 1.1	—	35.1 ± 0.4	6.0 ± 0.4	13.7 ± 0.3	—
36 h[d]	tr	0.5	30.0	tr	3.9	12.8	—	32.4	7.0	12.8	—
60 h[e]	0.3	0.7	25.2	tr	4.1	15.1	—	31.6	8.1	14.8	—
12°C	0.6 ± 0.2	1.3 ± 0.1	31.4 ± 2.1	tr-1.1	0.6 ± 0.1	10.9 ± 0.9	—	25.7 ± 0.8	9.6 ± 0.8	18.7 ± 0.2	tr-0.3
PG											
30°C	tr-0.3	1.6 ± 0.1	10.6 ± 1.0	43.1 ± 2.2	tr-0.2	3.1 ± 0.4	2.9 ± 0.2	18.3 ± 0.8	tr-0.5	21.5 ± 0.3	—
12 h	tr-0.3	1.0 ± 0.1	10.6 ± 0.5	44.3 ± 0.7	tr	3.5 ± 0.1	2.3 ± 0.3	17.5 ± 0.3	tr	21.4 ± 1.0	—
36 h	tr-0.2	tr-0.6	10.9 ± 0.6	43.6 ± 1.2	0.6 ± 0.1	5.5 ± 0.1	2.1 ± 0.3	15.7 ± 0.3	—	21.2 ± 0.5	—
60 h	tr-0.3	tr-0.6	12.6 ± 0.6	40.1 ± 0.6	0.4 ± 0.1	4.2 ± 0.3	2.3 ± 0.3	12.9 ± 0.3	—	26.9 ± 1.6	—
12°C	tr-0.5	1.2 ± 0.3	15.9 ± 1.1	35.5 ± 1.1	tr-0.2	4.0 ± 0.4	3.9 ± 0.2	9.9 ± 1.5	tr-0.4	28.5 ± 0.8	—
PE											
30°C	0.9 ± 0.1	3.4 ± 0.9	20.5 ± 0.5	tr-0.6	0.6 ± 0.2	26.9 ± 0.1	tr-1.0	28.0 ± 0.3	6.0 ± 0.1	12.4 ± 1.0	—
12 h	tr-0.7	2.6 ± 0.4	22.1 ± 2.9	tr-1.1	tr-0.4	30.6 ± 2.3	—	26.5 ± 0.7	5.2 ± 0.6	10.3 ± 0.8	—
12°C	0.7 ± 0.2	2.4 ± 0.1	17.7 ± 1.2	tr-0.6	0.3 ± 0.1	23.5 ± 0.9	tr-2.3	27.5 ± 0.5	10.1 ± 0.4	16.5 ± 0.4	—
DGTH											
30°C	—	tr	33.7 ± 0.2	tr-0.6	tr-0.3	2.9 ± 0.2	—	35.4 ± 0.1	9.7 ± 0.2	16.5 ± 0.5	tr-0.3
12 h	—	tr	32.1 ± 1.6	tr-0.5	1.3 ± 0.3	6.9 ± 0.2	—	32.5 ± 0.3	10.3 ± 0.3	15.9 ± 0.2	0.5 ± 0.1
36 h[d]	—	tr	36.5	0.6	tr-1.1	5.3	—	24.2	12.8	18.8	0.9
60 h[d]	—	tr	33.8	0.8	1.1	6.9	—	23.8	12.8	19.1	1.6
12°C	—	tr	33.3 ± 1.6	tr-0.6	0.3 ± 0.1	5.6 ± 0.2	—	18.2 ± 0.7	16.5 ± 0.1	22.8 ± 0.5	2.1 ± 0.3

[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.

[d] Sample number = 2.

[e] Sample number = 1.

lipid content occurring between 36 and 60 h represents a relatively small fraction of the total change (10 mol % decrease in MGDG and 8 mol % increase in DGDG) occurring during the approximate 100-h time required for acclimation.

DISCUSSION

The findings presented in this and the accompanying communication show that *Dunaliella*, like many other plants, does alter its lipid composition when chilled. It is now evident, based on the analyses included in this present study and others (6, 17), that the nature and the timing of the alterations which occur in response to low temperature stress differ for different membranes and different lipid classes. Thus, the acyl chain composition of chloroplast DGTH showed initial changes between 12 and 36 h after chilling while changes in PG commenced between 36 and 60 h, and changes associated with PC occurred later than 60 h after a shift in temperature. Compositional changes in microsomal phospholipids (see accompanying paper) were also temporally separated, but the major changes were observed at an earlier time (within 12 h after chilling) than were those of the chloroplast lipids.

Phospholipid molecular species analyses demonstrated the specificity that exists with respect to the distribution of acyl chain pairs within each lipid class. An awareness that chilling induced changes of a certain acyl chain within a particular lipid class generally involved only a few of the many molecular species could have been realized only by resolving the molecular species. For example, the fatty acid analysis showing the relative decrease in 18:2 of PC was irresolute, whereas the molecular species analysis demonstrated that 16:0/18:2 and 18:2/18:2 decreased at low temperature, but the other 18:2-containing species increased. This suggests an active and programmed retailoring of the phospholipid molecular species.

Several lines of evidence have suggested that molecular species alterations may be very important in bringing about significant fluidity-related changes in membranes and could play a crucial role in temperature acclimation. Inter- and intramolecular mixing of different acyl chains is known to have dramatic effects on the physical properties of lipids (4, 15, 16). These types of changes may not be detected by routine fatty acid analyses. It has been found that changes in the physical properties of *Tetrahymena* microsomal membrane lipids following a shift to low temperature were more closely correlated with molecular species alterations than with changes in acyl chain composition (5, 6).

A possible involvement of molecular species changes in temperature acclimation may also be inferred from certain findings which failed to demonstrate a relationship between membrane physical properties and acyl chain composition (13, 17). For example, investigations of *Nerium oleander* thylakoid membranes following a shift in growth temperature demonstrated that changes in membrane lipid physical properties, evaluated by ESR and Chl fluorescence, did not correlate with changes in acyl chain composition in a linear fashion (17). An analysis of lipid molecular species was not carried out in this study.

Chl fluorescence yield was used to follow changes in chloroplast membrane properties during the acclimation process of *Dunaliella*. It is postulated that heating the thylakoid membrane above a certain threshold temperature induces thermal denatur-

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

Cells were grown at 30°C or acclimated to 12°C or shifted to 12°C for 12, 36, or 60 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°C	30°C	30→12°C			12°C	30°C	30→12°C	12°C
		12 h	36 h	60 h			12 h	36 h	60 h			12 h	
16:0/14:1[a]	1.8 ± 0.1	2.0 ± 0.2	1.7 ± 0.1	2.2 ± 0.1	2.2 ± 0.4	0.6 ± 0.2	1.1 ± 0.1	0.4 ± 0.2	0.5 ± 0.4	0.9 ± 0.2	2.7 ± 0.4	2.6 ± 1.1	1.4 ± 0.2
16:0/14:2	3.1 ± 0.1	3.3 ± 0.5	2.4 ± 0.1	2.8 ± 0.2	4.2 ± 0.5	2.3 ± 0.8	3.3 ± 0.7	1.7 ± 1.3	1.5 ± 1.2	3.0 ± 0.6	12.5 ± 2.6	10.5 ± 3.1	7.4 ± 1.6
NI[b]	1.0 ± 0.3	2.2 ± 0.7	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	tr-0.3	0.5 ± 0.3	1.4 ± 0.2	1.2 ± 0.3	tr-0.3	0.9 ± 0.2	0.9 ± 0.2	1.9 ± 0.6
16:0/16:0	—[c]	—	—	—	—	—	—	—	—	—	—	—	—
18:0/14:1	—	—	—	—	—	—	—	—	—	—	tr[d]	tr	tr
18:0/14:2, 18:1/14:1	—	—	—	—	—	—	—	—	—	—	tr	tr	tr
16:0/18:1	2.0 ± 0.3	1.0 ± 0.1	3.0 ± 0.4	1.1 ± 0.1	3.2 ± 1.0	—	—	—	—	—	6.4 ± 0.5	5.0 ± 1.0	3.8 ± 0.2
18:1/16:1	—	—	—	—	—	[e]					—	—	—
16:0/18:2$^{\Delta 6,9}$	—	—	—	—	—						—	—	—
16:0/18:2$^{\Delta 9,12}$	48.2 ± 0.8	48.1 ± 0.4	51.9 ± 0.2	50.6 ± 0.1	31.9 ± 0.4	10.7 ± 1.2[e,f]	9.5 ± 0.4	10.5 ± 1.0	10.4 ± 0.8	12.4 ± 0.3	6.9 ± 0.1	8.5 ± 1.8	9.6 ± 1.3
18:2$^{\Delta 9,12}$/16:1	—	—	—	—	—	36.8 ± 1.4	33.5 ± 2.6	34.2 ± 0.4	26.4 ± 0.2	20.7 ± 1.5	—	—	—
16:0/18:3$^{\Delta 9,12,15}$	11.9 ± 0.5	11.4 ± 0.4	12.9 ± 0.4	15.1 ± 2.9	13.3 ± 0.5	tr	1.0 ± 0.1	1.7 ± 0.3	2.7 ± 0.5	2.4 ± 0.4	0.5 ± 0.1	0.7 ± 0.1	1.2 ± 0.3
18:3$^{\Delta 9,12,15}$/16:1	2.3 ± 0.6	1.8 ± 0.6	1.2 ± 0.1	0.4 ± 0.1	2.4 ± 0.1	45.5 ± 1.1	45.6 ± 0.3	48.4 ± 0.2	56.6 ± 1.8	57.6 ± 1.1	tr	tr	tr
NI	—	—	—	—	—	2.3 ± 0.5	3.9 ± 0.2	tr-1.0	tr-0.3	1.5 ± 0.1	—	—	—
18:1/18:1, 18:0/18:2	0.6 ± 0.1	tr-0.4	tr	tr	0.9 ± 0.6	—	—	—	—	—	5.8 ± 1.3	8.0 ± 2.7	7.3 ± 0.8
18:1/18:2$^{\Delta 9,12}$, 18:0/18:3	2.3 ± 0.2	4.0 ± 0.2	3.9 ± 0.5	3.7 ± 1.1	9.6 ± 0.7	—	—	—	—	—	18.7 ± 1.0	19.9 ± 0.4	21.4 ± 0.9
18:2$^{\Delta 9,12}$/18:2$^{\Delta 9,12}$	13.2 ± 1.1	14.6 ± 0.7	11.9 ± 0.1	10.3 ± 1.6	10.0 ± 0.5	—	—	—	—	—	28.0 ± 0.8	25.9 ± 3.3	27.8 ± 1.1
18:1/18:3$^{\Delta 9,12,15}$, 18:1/18:3$^{\Delta 6,9,12}$	2.9 ± 0.7	3.4 ± 0.2	4.7 ± 0.4	6.2 ± 1.1	7.2 ± 0.5	—	—	—	—	—			
18:2/18:3$^{\Delta 6,9,12}$	6.0 ± 0.3	5.3 ± 0.6	4.7 ± 0.4	5.3 ± 1.4	7.8 ± 0.7	—	—	—	—	—	8.7 ± 0.6	7.2 ± 1.6	9.5 ± 0.9
18:2/18:3$^{\Delta 9,12,15}$	1.0 ± 0.3	0.7 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	2.7 ± 0.5	—	—	—	—	—	2.1 ± 0.4	3.9 ± 1.8	6.6 ± 1.3
18:3$^{\Delta 9,12,15}$/18:3	1.1 ± 0.2	0.8 ± 0.1	tr-0.8	0.2 ± 0.1	1.5 ± 0.2	—	—	—	—	—	1.1 ± 0.4	1.3 ± 0.2	1.1 ± 0.2

[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] tr, trace.

[e] 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.

[f] This peak also contained 18:2/16:0 as established by positional determination of chloroplast PG acyl chains (19).

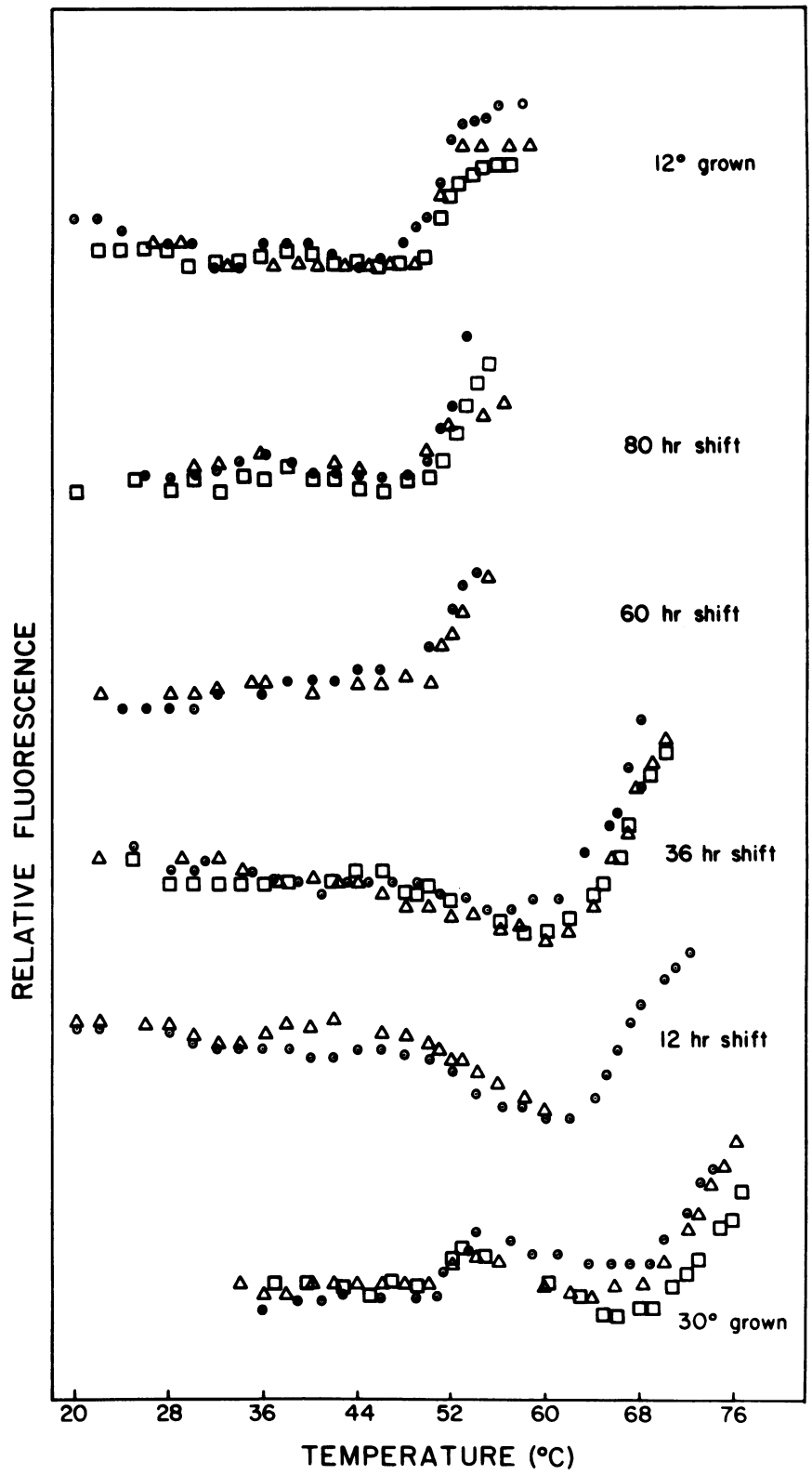

FIG. 1. Effect of chilling on the threshold temperature of Chl fluorescence (F_0) rise in whole cells. Cells were grown at 30 or 12°C or shifted from 30 to 12°C for the designated times prior to monitoring the temperature of enhanced fluorescence. Each symbol represents a separate experiment.

ation of proteins associated with the PSII reaction center (18) and a physical separation of the light-harvesting complex from the PSII core (1), blocking excitation energy transfer. This blockage results in a rise in Chl fluorescence. The temperature at which enhanced fluorescence occurs is an index of the thermal stability of the photosynthetic membrane and is related to the lipid environment in which the photosynthetic apparatus is embedded (2, 14, 17, 18).

When this technique was applied to our system, the downward shift in the threshold temperature which occurred between 36 and 60 h following a shift to low temperature (Fig. 1) closely correlated with the changes in the molecular species of PG (Table II). No other potentially variable parameters of lipid composition exhibited appreciable changes during this period. Much indirect evidence suggests that PG (having $16{:}1^{\Delta trans\text{-}3}$) is intimately associated with the photosynthetic apparatus and the organization of

chloroplast membranes (7, 8, 19). This being the case, it would not be altogether surprising to find that alterations in the molecular species composition exert profound effects on the thermal stabilty of the photosynthetic apparatus.

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