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CORRELATIONS BETWEEN THE THERMAL STABILITY OF CHLOROPLAST (THYLAKOID) MEMBRANES AND THE COMPOSITION AND FLUIDITY OF THEIR POLAR LIPIDS UPON ACCLIMATION OF THE HIGHER PLANT, *NERIUM OLEANDER*, TO GROWTH TEMPERATURE *

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The thermal stability of excitation transfer from pigment proteins to the Photosystem II reaction center of *Nerium oleander* adjusts by 10 Celsius degrees when cloned plants grown at 20°C/15°C, day/night growth temperatures are shifted to 45°C/32°C growth temperature or vice versa. Concomitant with this adjustment is a decrease in the fluidity of thylakoid membrane polar lipids as determined by spin labeling. The results are consistent with the hypothesis that there is a limiting maximum fluidity compatible with maintenance of native membrane structure and function. This limiting fluidity was about the same as for a number of other species which exhibit a range of thermal stabilities. Inversely correlated shifts in lipid fluidity and thermal stability occurred during the time course of acclimation of *N. oleander* to new growth temperatures. Thus, the temperature at which the limiting fluidity was reached changed during acclimation while the limiting fluidity remained constant. Although the relative proportion of the major classes of membrane polar lipids remained constant during adjustments in fluidity, large changes occurred in the abundance of specific fatty acids. These changes were different for the phospho- and galacto-lipids suggesting that the fatty acid composition of these two lipid classes is regulated by different mechanisms. Comparisons between membrane lipid fluidity and fatty acid composition indicate that fluidity is not a simple linear function of fatty acid composition.

Introduction

The temperature response of photosynthesis by leaves of higher plants may differ among species native to regions with contrasting thermal regimes or between leaves which have developed at different growth temperatures. Such differences are important in adapting or acclimating plants to tolerate and/or perform efficiently over a wide range of temperature since photosynthesis is among the most sensitive of plant processes to high temperature (for a review, see Ref. 1). The capacity of

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Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SL, sulphoquinovosyldiacylglycerol; 16:0, palmitic acid; 16:1, palmitoleic acid; 16:2, hexadecadienoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:12, linoleic acid; 18:3, linolenic acid.

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a leaf to tolerate high leaf temperature without damage to the photosynthetic apparatus has been correlated with differences in the chloroplast membranes [2,3,4]. Membrane-associated reactions such as Photosystem II electron transport and photophosphorylation are more stable to high temperature in membranes from plants adapted to or acclimated to high temperature than in membranes from plants adapted to or acclimated to lower temperatures [2,5,6]. Studies of chlorophyll fluorescence from intact leaves have shown that at high temperatures there is a change in the structural arrangements which normally permit the efficient transfer of excitation energy from chlorophyll-proteins to the Photosystem II photochemical reaction centers [7,8]. As a result of these changes there is an increase in chlorophyll fluorescence with heat damage, and the temperature at which this increase in fluoresence occurs provides an index of the thermal stability of the chloroplast membrane [4,7]. Other studies have shown that irreversible inhibition of the photosynthetic capacity of intact leaves occurs when they are heated to temperatures above this threshold for fluorescence increase [3]. This fluorescence index has proved useful in ranking species according to their high temperature tolerance [9,10], and in studies of the acclimation of a species to different growth temperatures [11].

The differences in membrane thermal stability might be attributed to differences in the proteins or lipids of the chloroplast membranes. Pearcy [12] has shown that the fatty acids of lipids from leaves of the higher plant *Atriplex lentiformis*, which developed at high temperature, have fewer double bonds per fatty acid than do the corresponding lipids from leaves which developed at low temperature. Other studies have shown similar differences in lipid composition among species which are native to hot desert or cool maritime climates [3]. A number of studies have examined changes in membrane lipid ordering over the low temperature range 0–15°C [13,14]. However, no studies have examined the physical properties of membrane lipids over the range where high temperature damage occurs (typically 40–50°C), nor has there been any attempt to correlate changes in membrane lipid structure with membrane function in the high temperature range.

In this study we have taken advantage of the capacity of fully expanded leaves of the evergreen shrub, *Nerium oleander* to acclimate to contrasting growth regimes. Leaves from plants grown at high (45°C) or at low (20°C) temperature had higher rates of photosynthesis at the growth temperature in comparison to leaves acclimated to growth at the contrasting temperature regime. Leaves from high temperature grown plants also had a higher temperature optimum for photosynthesis, and could tolerate higher temperatures before being damaged by high temperature than could leaves from low temperature grown plants. Leaves of plants transferred from low to high or high to low temperature adjust physiologically over the course of several days becoming physiologically similar to leaves of other plants which had developed at the new growth regime. Details of the photosynthetic performance characteristics of the photosynthetic apparatus of these leaves are reported elsewhere [5,15]. In this paper we examine the relation between changes in chloroplast membrane stability induced by growth temperature and the physical properties of chloroplast membrane lipids. Stability of the chloroplast was assayed by chlorophyll fluorescence, and the physical properties of chloroplast membrane lipids, determined by spin labeling. The results suggest a physical basis for differences in the apparent thermal stability of the photosynthetic membranes of plants from differing thermal regimes. We further examine the changes in fatty acid composition which accompany these changes in lipid properties.

Materials and Methods

Plant material. Plants of a single clone of *Nerium oleander* were grown in controlled growth facilities with either a 20°C/15°C or 45°C/32°C diurnal temperature regime. *Atriplex lentiformis* was grown at 20°C/15°C or 40°C/25°C temperature regimes and *Atriplex sabulosa* was grown at 20°C/15°C. *Tidestromia oblongifolia* was either grown in controlled growth facilities at 45°C/32°C or collected from its natural habitat in Death Valley National Monument. *Spinacea oleraceae* was grown in a green house. Transfer experiments were started at the beginning of a dark period and harvested after one or more days exposure to be the new thermal

regime. Harvests were made at the beginning of the light period.

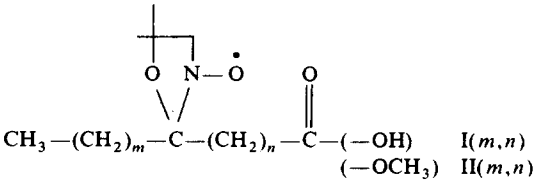
Fluorescence studies. Fluorescence versus temperature measurements were made by enclosing a leaf in a water-jacketed and stirred cuvette essentially as described by Ref. 7. The temperature of the cuvette was increased at 1 Celsius degree/min using a temperature controlled circulator. The temperature was measured by a thermocouple appressed to the leaf surface, and fluorescence was monitored through a narrow band-pass interference filter (λ max = 690 nm). Fluorescence excitation was $1 \cdot 10^{-11}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1} broad-band blue light (Corning 9782 filter). The fluorescence and temperature signals were displayed on an *x-y* recorder yielding a fluorescence-versus-temperature curve. Air within the cuvette was humid. In the experiments described here the leaves remained attached to the plant. Small discs cut from the leaf yielded similar results.

Isolation of thylakoids. All steps were carried at 4°C. 10 to 15 g of leaves, with mid-ribs removed, were cut on a glass plate into 1 to 2 mm thick slices using a razor blade. They were then vigorously homogenized in a motor and pestle with 100 ml of mixture consisting of 0.4 M sorbitol, 0.1 M Tricine-NaOH (pH 7.6), 5 mM $MgCl_2$, 50 μM ascorbate and 0.25% (w/v) fatty acid-free, bovine serum albumin. The brie was filtered through Miracloth (Chicopee Mills, Milltown, NJ) and centrifuged at $10000 \times g$ for 15 min. The chloroplasts were resuspended in 5 mM NaCl with a camel hair brush, taking care not to resuspend the starch grains, and were pelleted at $20000 \times g$ for 10 min. This step was repeated once. Some contaminating mitochondrial membranes were detected in these thylakoid preparations (see below).

Lipid extraction and analysis. All solvents were redistilled. Lipids were extracted from the thylakoid pellet according to Folch et al. [16]. A portion of the total lipid was analyzed for fatty acids. 50 mg of lipid, in a small volume of $CHCl_3$, was loaded on to a column of 6 g of activated silicic acid suspended in $CHCl_3$ and eluted with 60 ml of $CHCl_3$, to remove most of the pigments. Polar lipids, used for measurements of fluidity, were subsequently eluted from the column with 60 ml of CH_3OH. For thin-layer chromatography (TLC), a column (6 g) was sequentially eluted with 60 ml of $CHCl_3$ (pigments and neutral lipids), 190 ml of $(CH_3)_2CO$ (galacto- and sulpho-lipids) and 60 ml of CH_3OH (phospholipids).

Thin-layer chromatography, and preparation and analysis of methyl esters. TLC of thylakoid phospholipids was performed in two dimensions according to Ref. 17. Separation of galactolipids and sulpholipid was by one-dimensional TLC using solvent 2 of Ref. 17. Methyl esterification and gas chromatography were performed according to Ref. 17. Heptadecanoic acid (17:0) was used as an internal standard to determine the relative amounts of each lipid.

Spin labeling and ESR spectroscopy. Liposomes were prepared from polar lipids deposited onto the surface of a glass tube by evaporating the solvent ($CHCl_3$) in a stream of N_2; residual solvent was removed under vacuum, and the lipid was dispersed in 0.1 M Tris-HCl buffer (pH 7.2), containing 5 mM EDTA (10 to 15 mg lipid per ml) by brief sonication. The liposomes were labeled with the *N*-oxyl-4,4-dimethyloxazolidine derivative of keto fatty acids or methyl esters with the general formulae



I(m,n)
II(m,n)

where the subscripts m and n indicate the position of substitution of the oxazolidine group. A motion parameter τ was calculated from the relationship

$$\tau = 6.45 \cdot 10^{-10}\left[(h_0/h_{-1})^{1/2} + (h_0/h_{+1})^{1/2} - 2\right] W_0$$

where h_{+1}, h_0 and h_{-1} refer to the height of the low-, mid- and high-field lines, respectively, and W_0 the width, in gauss, of the midfield line of the first derivative ESR spectrum. Spectra were recorded on a Varian E112 spectrometer fitted with a temperature-controlled cell housing. The temperature was measured with a copper-constantan thermocouple and was controlled to ± 0.05°C by a model TCm 20 temperature control unit (Deltron Pty Ltd., Sydney, Australia). The

order parameter S_n was calculated from the separation of inner and outer extrema as described by Gaffney [18].

Results and Discussion

Temperature stability

The temperature versus fluorescence course for intact leaves of *N. oleander* grown at either 20 or 45°C day temperature are shown in Fig. 1. The sharp increase in fluorescence which occurs as the temperature of the leaf is increased is most likely the result of denaturation of proteins of the light-harvesting pigment system of the membranes [8]. The threshold temperatures for this denaturation can be estimated as 43 and 53°C from Fig. 1 for the 20°C- and 45°C-grown plants, respectively. This result indicates that at least the integrity of the light-harvesting pigment system in chloroplast membranes is stable to a temperature 10 Celsius degrees higher in high temperature-acclimated than low temperature-acclimated plants.

Spin labeling and changes in membrane lipid fluidity

First derivative spectra of label II(5,10) in chloroplast thylakoid membranes and in liposomes prepared from polar lipids of these membranes are compared in Figs. 2A and 2B. The spectrum for the polar lipids (Fig. 2B) shows three well defined, symmetrical absorption lines which indicate the label is undergoing relatively rapid and tumbling motion, similar to the motion of this label in castor oil [19]. In contrast, the spectra of the probe in membranes has a broader shoulder of absorption on the low field side of the +1 line and a relatively narrow shoulder on the low field side of the −1 line (see Fig. 2A). Subtraction of the liposome spectrum from the membrane spectrum was adjusted, with the aid of a computer, to remove absorption due to the rapid tumbling motion of the label giving the narrow component of the +1 and −1 lines. The resulting spectrum (Fig. 2C) shows considerable broadening typical of label adsorbed to and immobilized on the hydrophobic surfaces of proteins as shown by Birrell et al. [20] with chromatophores isolated from *Rhodopseudomonas sphaeroides*.

Comparative measurements of both spin label motion and order, with membranes and liposomes

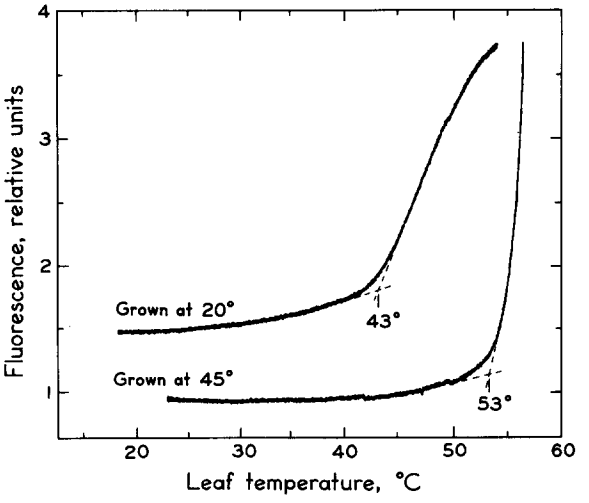


Fig. 1. Change in the relative intensity of chlorophyll fluorescence in whole, attached leaves of *N. oleander* as a function of temperature. The temperature of the fluorescence increase was determined as the point of intersection of the lines extending the two, almost linear portions of the curves, as shown.

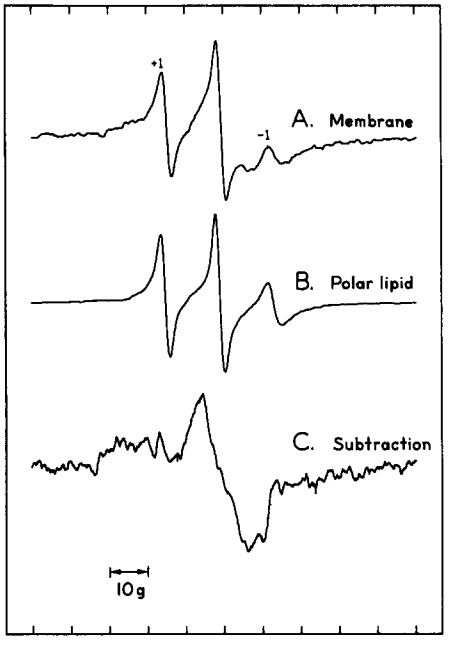


Fig. 2. First derivative spectra of label II(5,10) with (A) thylakoid membranes, (B) polar lipids and (C) spectrum A minus spectrum B. All spectra were adjusted to equal height. For subtraction, the peak height of B was adjusted to remove the narrow component of the low (+1) and high-field (−1) peaks of A.

TABLE I

COMPARISON OF THE ORDER PARAMETER (S_n) AND MOTION (τ_o) FOR SPIN LABELS IN THYLAKOID MEMBRANES AND POLAR LIPID SUSPENSIONS FROM *N. OLEANDER* GROWN AT WARM AND COOL TEMPERATURES

Data are presented as S_n values determined with I(12,3) acid and τ values determined with II(5,11).

Temp. (°C)	Growth at 20°C		Growth at 45°C	
	S_n	τ (s) ($\times 10^{10}$)	S_n	τ (s) ($\times 10^{10}$)
Thylakoid membranes				
20	0.633±0.002	108 ±8	0.675±0.003	120 ±14
30	0.610±0.001	50 ±2	0.631±0.004	53 ± 4
40	0.559±0.003	32 ±1	0.579±0.005	33 ± 1
50	0.516±0.003	22 ±0.5	0.543±0.003	22 ± 1
Polar lipids				
20	0.599±0.002	22 ±0.6	0.607±0.002	28 ± 0.8
30	0.539±0.002	15.6±0.3	0.535±0.004	20.9± 0.3
40	0.470±0.003	11.2±0.2	0.470±0.004	14.5± 0.4
50	0.396±0.002	8.4±0.3	0.397±0.012	10.6± 0.2

from plants grown at warm and cool temperatures, are shown in Table I. At corresponding temperatures the values of τ, calculated from spectra of II(5,10) with membranes, are 3- to 5-fold greater than those determined with the corresponding liposomes of the polar lipids (Table I). As shown above (Fig. 2), the immobilization of spin label probe by components of the intact membrane distorts the ESR spectrum. The apparently restricted motion in the intact membrane is largely an artefact of this distortion, although some restriction of lipid motion by boundary-layers of intrinsic membrane proteins may occur [21]. Since a spectrum characteristic of a relatively fluid environment could be separated from the composite spectrum of label with membranes (see Fig. 2) we assume that the membrane lipids are of similar fluidity to that of the corresponding liposomes at the same temperature, and for the purpose of these comparative studies we consider that the values of S_n and τ determined with liposomes of purified polar lipids from the membrane provide a satisfactory estimate of fluidity in the lipid domains of the intact membrane.

As shown in Table I, the lower growth temperature resulted in synthesis of lipid with increased spin label motion (decreased τ). The order parameter (S_n) was also somewhat lower (indicating greater motion at 20°C in lipid from plants acclimated to growth temperature, but this difference was insignificant when S_n was measured at >40°C. While both parameters (τ and S_n) provide comparative measures of membrane fluidity, they probe different regions of the membrane. The

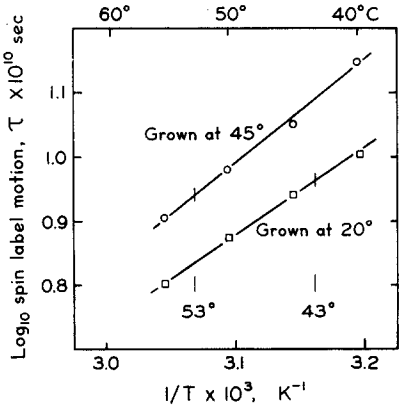


Fig. 3. Arrhenius-type plots of the change in the logarithm of spin label motion as a function of the reciprocal of the absolute temperature. The data is for label II(5,10) with polar lipids from plants grown in the temperatures indicated. The 53 and 43°C represent the threshold temperature for denaturation (T_d), determined as described in Fig. 1.

label I(12,3) used to measure S_n probes a location relatively close to the surface of the bilayer whereas II(5,10) used to measure τ probes a location near the center of the bilayer [21]. The data presented in Table I thus indicate that at any temperature between 20 and 50°C the membrane lipids from plants grown at 20°C are more fluid than those of plants grown at 45°C and that the motion parameter τ is a more sensitive method of detecting the change in fluidity than measurement of S_n.

The logarithm of the motion parameter is linearly related to the reciprocal of the absolute temperature [23]. The straight lines representing this relationship for the polar lipids from both high- and low-temperature-grown plants is shown in Fig. 3, for the temperature range of 40 to 55°C and these can be used to interpolate the value of τ at any temperature within this range. The linearity of the Arrhenius-type plots shown in Fig. 3 also indicates that there is no phase transition affecting the molecular ordering of membrane lipids at temperatures which correspond to the threshold for irreversible damage to the photosynthetic apparatus at high temperature. However, since fluidity increases with decreasing growth temperature we are attracted to the view that for thylakoid membranes an upper limit exists for lipid fluidity which is compatible with maintenance of structural and functional integrity as shown by McElhaney and Souza [24] for *E. coli*.

Correlations between fluidity and thermal stability

If the temperature thresholds for denaturation of the respective chloroplast membranes, as determined in Fig. 1, are used to estimate the vlaue of τ at the denaturation temperature (from Fig. 3) we arrive at the same value ($(8.6–8.8) \cdot 10^{-10}$ s) for the two preparations. This result implies a direct relationship between the temperature for functional denaturation of chloroplast membrane proteins and the molecular motion or fluidity within the hydrophobic region of the membrane.

Similar studies with additional species have been conducted, and the results are summarized in Table II. Shown are the species, the growth conditions, the threshold temperature for denaturation (T_d), and the motion parameter at the threshold temperature (the critical motion parameter, τ_d). The value of τ_d ranges between 7.9 and $10.0 \cdot 10^{-10}$ s, but paired determinations with the same species acclimated to different temperatures are within $0.2 \cdot 10^{-10}$ s. This result indicates that for a given species the value of τ_d is fairly constant. There are somewhat larger differences between species, however. One might expect this result, since other factors such as differences in the primary structure of the proteins of the reaction center complex must be considered when comparing across species boundaries. The value of τ_d for all of these species is nevertheless quite similar with a mean of $(9.0 \pm 0.8) \cdot 10^{-10}$ s. The standard

TABLE II

THRESHOLD TEMPERATURE FOR DENATURATION (T_d) AND THE CORRESPONDING MOTION OF THE SPIN LABEL II(3,12) IN LIPOSOMES OF CHLOROPLAST POLAR LIPIDS AT 45°C (τ_{45}) AND AT T_d (τ_d)

Species	Growth temperature (°C)	T_d (°C)	τ_{45} (s) ($\times 10^{10}$)	τ_d (s) ($\times 10^{10}$)
Spinacea oleraceae	[a]	43	8.9	9.6
Atriplex sabulosa	20/15	45	10.7	9.5
Atriplex lentiformis	20/15	44	9.4	9.8
	40/25	50	11.9	10.0
Tidestromia oblongifolia	[b]	48.5	8.9	7.9
	45/32	52.3	10.7	8.0
Nerium oleander	20/15	43	8.5	8.8
	45/32	53	10.9	8.6

[a] Greenhouse approx. 25°C/20°C.
[b] Collected in Death Valley, CA, Dec. 17, 1979, approx. 20°C/5°C.

deviation corresponds to about ±3 Celsius degrees if the variation is considered in terms of the threshold temperature (estimated from the slope of the lines in Fig. 3). The error in determination of T_d is about ±1 Celsius degree.

Acclimation experiments

Another perspective from which to examine the relationship between thermal stability and membrane lipid fluidity is provided by kinetic studies of acclimation followed a sudden change in growth temperature. *N. oleander* plants grown at either 45 or 20°C can be transferred to the reciprocal growth regime, and over a period of time these plants will acclimate to their new growth conditions. After 10–14 days leaves which had been transferred to a new growth regime were functionally indistinguishable from leaves which had developed at that growth regime [15]. Studies of the change in T_d in τ at a given temperature (45°C) and τ_d during reciprocal transfer experiments are shown in Fig. 4. The threshold temperature (T_d) adjusts upward or downward upon transfer. This process is very rapid, with acclimation approximately half complete after one day. Rapid and corresponding changes in the value of τ measured at 45°C were also observed. These indicate that during acclimation lipid fluidity decreases upon transfer from low to high temperature and increased upon transfer from high to low temperature. This adjustment is such that the interpolated, critical lipid motion parameter (τ_d) was nearly constant at $(8.6 \pm 0.2) \cdot 10^{-10}$ s for all determinations of τ_d with this species. The deviation corresponds to less than ±1 Celsius degree in the value of T_d.

These studies do not specifically address the possibility that changes in the stability of the membrane is in part based upon changes in the protein components of the membrane. While this possibility cannot be eliminated, the strong correlations reported here between fluidity and thermal stability with acclimation, indicate a strong probability that membrane lipid fluidity plays an important role in modulating the thermal stability of the chloroplast membrane. Details of the denaturation of the membrane proteins are not known; however, water structure also plays a role since substitution of ^2H_2O for H_2O results in a

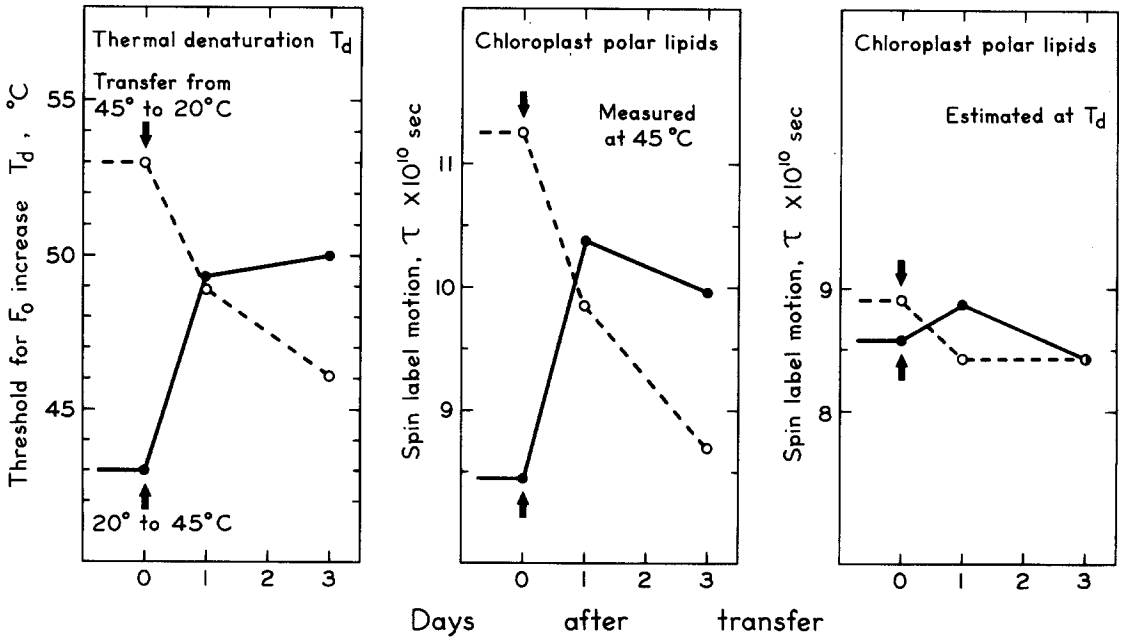


Fig. 4. Changes in the thermal stability, motion parameter (τ) of polar lipids at 45°C and the critical motion parameter (τ_d) for chloroplast membrane stability, determined after transfer of *N. oleander* plants from growth at 45°C to growth at 20°C and vice versa. The data points were determined from data set analogous to those of Figs. 1 and 3.

TABLE III

CHANGES IN THE ACYL FATTY ACID COMPOSITION OF CHLOROPLAST MEMBRANE LIPIDS OF *N. OLEANDER* DURING ACCLIMATION FROM GROWTH AT 20°C/15°C TO GROWTH AT 45°C/32°C

Lipid species		Fatty acids (% of total)					
		16:0	16:1	18:0	18:1	18:2	18:3
Total (100%)	control	15.2±1.5	<1.0	2.2±0.4	5.8±1.1	12.4±1.3	64.7±1.4
	1 day	16.6±1.5	<1.0	2.8±0.3	7.8±0.4	12.5±1.0	60.3±1.5
	14 day	19.0±1.7	<1.0	4.2±0.7	11.1±0.8	15.1±1.3	50.4±2.1
Monogalactosylacylglycerol (51%)	control	1.7±0.9	<0.5	0.5±0.1	1.1±0.3	3.2±0.7	93.6±0.9
	1 day	3.0±0.4	<0.5	0.8±0.1	2.5±0.4	7.9±0.8	85.5±1.0
	14 day	3.0±0.6	<0.5	0.9±0.2	4.9±0.5	14.5±1.8	76.6±1.9
Digalactosylacylglycerol (30%)	control	14.9±1.3	<0.5	3.8±0.6	2.1±0.3	2.6±0.7	76.6±2.1
	1 day	17.4±1.0	<0.5	4.7±0.4	2.5±0.6	4.5±0.3	70.9±1.5
	14 day	20.4±1.6	<0.5	7.8±0.4	6.3±1.6	13.5±1.9	52.0±2.3
Sulphoquinovosylacylglycerol (7%)	control	37.7±2.9	<0.5	3.1±0.5	12.2±2.7	7.0±0.9	40.0±3.3
	1 day	41.9±1.8	<0.5	3.2±0.4	14.4±1.2	8.2±1.3	32.3±1.7
	14 day	46.5±2.4	<0.5	4.6±0.8	18.2±2.0	9.7±1.4	20.4±2.6
Phosphatidylglycerol (6%)	control	27.6±1.9	18.1±3.4	1.7±0.4	33.2±3.3	12.9±2.7	6.6±1.3
	1 day	38.5±2.4	15.8±0.6	2.6±0.5	29.8±1.6	8.8±1.0	4.6±0.6
	14 day	43.5±2.5	10.2±1.0	2.5±0.7	39.6±0.9	3.1±0.7	1.3±0.2
Phosphatidylethanolamine (3%)	control	16.5±2.5	<0.5	2.4±0.7	7.4±2.0	57.8±3.4	15.9±2.9
	1 day	22.2±1.3	<0.5	3.0±0.2	11.2±1.5	46.2±1.9	17.5±0.7
	14 day	20.0±3.1	<0.5	3.6±0.9	21.1±2.1	36.0±3.7	19.1±2.4
Phosphatidylcholine (1.8%)	control	16.2±2.2	<0.5	3.1±0.5	11.5±1.9	49.5±2.2	19.6±4.5
	1 day	24.5±2.9	<0.5	5.2±0.3	22.2±1.4	30.1±0.9	18.2±2.3
	14 day	27.8±5.5	<0.5	5.1±0.5	29.4±3.2	20.0±3.6	17.9±1.3
Phosphatidylinositol (1.2%)	control	42.5±1.9	<0.5	3.3±0.3	6.8±2.9	26.5±3.3	20.7±3.5
	1 day	46.9±0.3	<0.5	3.4±0.4	9.9±0.9	21.7±0.8	18.2±0.4
	14 day	44.8±3.5	<0.5	3.5±1.0	13.4±0.7	17.2±2.2	21.1±3.2

stabilization of the membrane which is additive to that related to acclimation and correlated here with changes in lipid fluidity [25].

Compositional changes

The relative proportion of the neutral lipid, galacto-lipid and phospholipid classes did not alter significantly during acclimation (data not shown). However, there were large changes in the fatty acid composition of these lipids. Table III gives the fatty acid composition of both the thylakoid total lipid extract and of the various lipid classes isolated from plants grown initially at 20°C, then for 1 day and 2 weeks after being transferred to 45°C. Acclimation is complete after 2 weeks [15]. The data show that thylakoid lipid composition of *N. oleander* is similar to that of other higher plants [26,27]. It is reported, however, that the thylakoid membranes of spinach do not contain phosphotidylethanolamine (PE) [28]. Approx. 5% contamination of the chloroplast membranes with mitochondrial membranes which are about 50% PE and 30% PC [28] could account for the PE and some of the PC present in these extracts. Cytochrome oxidase activity, a marker for mitochondrial membranes [29] was detected in these preparations. This minor contamination could not be removed by differential centrifugation procedures. Measurements of the fatty acid composition of monogalactosyldiacylglycerol (MGDG), digalactosylacylglycerol (DGDG), sulphoquinovosylacylglycerol (SL) (Table III) would not be affected by this contamination since these lipids are exclusive to the chloroplast [29]. Those for, PC, PE and phosphatidylinositol (PI) include other cellular membranes in addition to the chloroplast membranes. These contaminants are of minor proportion and should not have large effects on the measured fluidity.

After acclimation to high growth temperature the proportion of linoleic acid (18:3) decreases in the total lipid extract, due mainly to large decreases of this acid in the MGDG, DGMG and SL fractions, in which 18:3 predominate. The palmitic (16:0), linoleic (18:2) and oleic acid (18:1) in these fractions increase during acclimation to high temperature. The major changes in the non-chloroplast phospholipids is an increase in 18:1 and 16:0 and a decrease in 18:2. Except for the phosphatidylglycerol fraction, 18:3 changes little in the phospholipids during acclimation to high temperature. Phosphatidylglycerol is the only lipid to contain significant palmitoleic acid (16:1). This acid decreases rapidly with acclimation to high temperature. Compositional changes similar in direction to those observed 2 weeks after transfer are apparent after 1 day at 45°C. For MGDG about 50% of the compositional change observed at 14 days, is complete after 1 day, while rapid changes also occur in phosphotidylcholine (PC) and phosphatidylglycerol (PG). Rapid changes in the composition of MGDG also appear to play a central role in acclimation of blue-green alga *Anabena variabilis* to changes in growth temperature [30].

The decrease in abundance of polyunsaturated fatty acids with increasing growth temperature is not simply the result of an inhibition of desaturation by a single fatty acid synthetase system. Such a mechanism cannot explain the four-fold increase in 18:2 in the galactolipids while the same acid decreases in the phospholipids during acclimation. It is also inconsistent with a decrease in 18:3 in the galacto- and sulpho-lipids while the level of this acid remains relatively constant in PE, PI and PC. To account for these various changes it is necessary to consider metabolism of fatty acids associated with the galactolipids distinct from that of these phospholipids. Consistent with this view is evidence of Appleby et al. [31] showing that the alga *Chlorella* has two independent sites of synthesis for 18:3 involving either PC or MGDG. Furthermore the existence of two sites of synthesis of phosphatidic acids and diacylglycerols in leaves, namely the endoplasmic reticulum and the chloroplast [32], might allow acylations generating phospholipids and galactolipids to proceed from separate pools of fatty acids. It appears that chloroplasts are autonomous for galactolipid synthesis [33] whereas they cannot make phospholipids other than phosphatidic acid [34]; thylakoid phospholipids presumably being imported from the endoplasmic reticulum. Therefore, if the fatty acid pool in the endoplasmic reticulum is largely separate from that in the chloroplast, different responses of phospholipid composition, compared to that of the galactolipids, could occur when leaf temperature changes.

The observation that the fatty acid composition of MGDG, PC and PG changes more rapidly than in other lipid species, after plants are transferred to 45°C, is consistent with their apparent central role in lipid metabolism. Thus, [1-^{14}C]acetate and $^{14}CO_2$ feeding experiments (see, for example, Ref. 33), invariably show that of the major lipid classes the acyl moieties of PC, PG and MGDG are the most rapidly labelled.

Fatty acid composition and membrane lipid fluidity

The correlation between the change in fatty acid composition and fluidity indicates that the decrease in membrane lipid fluidity is due to a decrease in the abundance of polyunsaturated fatty acids as might be anticipated [35]. The few studies on mixed membrane lipids show that fluidity increase with unsaturation. For example, NMR studies of the longitudinal relaxation times of the carbon atoms of the acyl chains in model systems of glycolipids [36] show that fluidity near the terminal methyl groups increases with the introduction of a double bond and increases further with additional unsaturation. However, fluidity is probably also influenced by the interaction between lipid head groups [37]. In this study, the changes in fluidity after one day as compared to that after two weeks, is much greater than the corresponding change in unsaturation of the acyl lipids over this period of time. Also, no changes in the head groups composition were detected. The quantitative change in lipid fluidity are, thus, not simply proportional to either the changes in fatty acid composition or lipid classes. A similar conclusion may be drawn from studies of changes in lipid fluidity and composition of the protozoan *Tetrahymena* during acclimation to contrasting growth temperature [38]. The non-linear relation between fluidity and composition might be explained if fluidity were more sensitive to the composition of particular lipid classes, such as MGDG or PG which change more rapidly than other lipid classes (Table III). It is also possible that lipid fluidity may depend upon acyl lipid composition as a function of the position of esterification on the glycerol moiety of the lipid molecules. These possibilities were not examined in the present study. Transient over-reaction of fatty acid composition during acclimations as noted with MGDG of *Anabena variabilis* [30], was not observed with *N. oleander*.

Conclusions

These results, particularly those shown in Fig. 4, suggest that lipid fluidity plays a significant role in determining the thermal stability of chloroplast thylakoid membranes in vivo, and that environmentally induced changes in fluidity appear to underlie the changes in thermal stability which occur with acclimation of plants to different growth regimes. These changes in fluidity are consistent with the changes in the fatty acid composition of the acyl lipids. Rapid adjustments in thermal stability of the membranes, the fluidity of the polar lipids and fatty acid composition occur upon a step transfer from one growth condition to another. The changes in overall fatty acid composition, however, do not seem to be directly proportional to the changes in fluidity. Either fluidity is dominated by a specific lipid class or the fluidity is not a direct function of fatty acid composition. The specific changes in fatty acid composition appear to be inconsistent with a single mechanism controlling the fatty acid synthesis of chloroplast lipids. Control of the fatty acid composition of the phospholipids PC, PE and PI, appear to be regulated by a separate mechanism from that which regulates the composition of the galacto- and sulpho-lipids of the chloroplast. The lipids which change most rapidly, MGDG, PC and PG are those which have been shown in other studies (see, for example, Ref. 34) to be the points of entry for newly synthesized fatty acids into the chloroplast lipid pool.

Other work has demonstrated that adjustments in lipid composition occur when higher plants acclimate to temperature [12]. This study establishes further correlations between these changes in composition and changes in membrane fluidity which appear to be linked to a significant physiological event, namely the threshold temperature for thermal denaturation of chloroplast membrane function. Other studies [2] indicate that this functional denaturation is one of the most sensitive plant functions to high temperature damage. The lipid properties of chloroplast membranes therefore appear to play a significant role in adapting

or acclimating plants to contrasting thermal regimes.

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