Plant Physiol. (1989) 90, 1134–1142
0032-0889/89/90/1134/09/$01.00/0

Received for publication November 22, 1988
and in revised form March 12, 1989

Enhanced Thermal Tolerance of Photosynthesis and Altered Chloroplast Ultrastructure in a Mutant of *Arabidopsis* Deficient in Lipid Desaturation[1]

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ABSTRACT

A mutant of *Arabidopsis thaliana*, deficient in activity of the chloroplast *n*-6 desaturase, accumulated high levels of $C_{16:1}$ and $C_{18:1}$ lipids and had correspondingly reduced levels of polyunsaturated lipids. The altered lipid composition of the mutant had pronounced effects on chloroplast ultrastructure, thylakoid membrane protein and chlorophyll content, electron transport rates, and the thermal stability of the photosynthetic membranes. The change in chloroplast ultrastructure was due to a 48% decrease in the amount of appressed membranes that was not compensated for by an increased amount of nonappressed membrane. This resulted in a net loss of 36% of the thylakoid membrane per chloroplast and a corresponding reduction in chlorophyll and protein content. Electrophoretic analysis of the chlorophyll-protein complexes further revealed a small decrease in the amount of light-harvesting complex. Relative levels of whole chain and protosystem II electron transport rates were also reduced in the mutant. In addition, the mutation resulted in enhanced thermal stability of photosynthetic electron transport. These observations suggest a central role of polyunsaturated lipids in determining chloroplast structure and maintaining normal photosynthetic function and demonstrate that lipid unsaturation directly affects the thermal stability of photosynthetic membranes.

The photosynthetic membranes of higher plants contain an unusually high proportion of polyunsaturated fatty acids. Depending on the plant species, trienoic acids ($C_{16:3}$ and $C_{18:3}$) and dienoic acids ($C_{16:2}$ and $C_{18:2}$) may account for 85% of the total fatty acids found within the chloroplast (11). While the functional significance of polyunsaturated fatty acids in thylakoid membranes is poorly understood, studies focusing on MGD,[2] the most abundant chloroplast lipid, indicate that the physical properties of this lipid are largely determined by its acyl chain composition (23). MGD accounts for up to 50% of the thylakoid lipid matrix and is characterized by its highly unsaturated acyl chain substituents which typically contain more than 5.5 *cis* double bonds per molecule (23). In contrast with saturated MGD, which forms conventional bilayer structures when dispersed in aqueous systems, highly unsaturated MGD forms hex-II micelles. The tendency of MGD to form hex-II structures has been suggested to aid in the packing of Chl-protein complexes into the thylakoid membranes (23). Furthermore, very small changes in the degree of unsaturation cause MGD to undergo a liquid-to-gel phase transition at room temperatures (10). Thus, the high proportion of polyunsaturated fatty acids in MGD may be an important factor in establishing and maintaining photosynthetic membrane function. Indeed, the acyl composition of MGD is under developmental control during chloroplast biogenesis (8) and changes in chloroplast lipid unsaturation have been shown to accompany whole plant acclimation to temperature extremes (24).

To examine the role of polyunsaturated fatty acids in chloroplast membranes we have isolated a number of mutants of *Arabidopsis thaliana* that have leaf lipids with altered acyl composition (28). Physiological analysis of a mutant deficient in synthesis of trienoic fatty acids did not reveal significant changes in photosynthetic properties but did demonstrate that trienoic acid levels influence chloroplast size and number (17). The properties of the recently isolated *fadC* mutant are described here. This mutant is deficient in the activity of the chloroplast *n*-6 desaturase, which normally desaturates $C_{16:1}$ and $C_{18:1}$ (4). As a consequence, the proportion of polyunsaturated fatty acids in the photosynthetic membranes of the *fadC* mutant is reduced to 51% of wild-type levels. The results presented here indicate that polyunsaturated fatty acids are important for thylakoid membrane organization, composition, and function. In addition, our results indicate that lipid polyunsaturation is an important determinant of the thermal stability of photosynthetic membranes in *Arabidopsis*.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The mutant line LK3 was isolated from the Columbia wild type of *Arabidopsis thaliana* (L.) Heynh. as previously described (17). The mutant carries a defective allele of a locus,

[1] This work was supported in part by grants from the U.S. Department of Agriculture (87-CRCR-1-2507), the U.S. Department of Energy (DE-AC02-76ER01338), and the Mcknight Foundation.

[2] Abbreviations: MGD, monogalactosyl-diacylglycerol; $C_{x:y}$, a fatty acyl group containing *x* carbons with *y* double bonds; *fad*C, symbol for a gene controlling activity of an *n*-6 desaturase; *fad*D, symbol for a gene controlling activity of an *n*-3 desaturase; F_o, initial fluorescence; F_v, variable fluorescence; F_m maximum fluorescence; fr wt, fresh weight; CPI, PSI reaction center (P700); CPI*, band containing CPI and the light-harvesting complex of PSI; CPII, band containing monomers of the light-harvesting complex of PSII; CPII*, band containing oligomeric LHCII; CP43 and CP47, bands containing PSII Chl *a* core complexes; FP, band containing free pigment; LHCP, light-harvesting Chl-protein complex.

designated *fadC*, which is required for the desaturation of $C_{16:1}$ and $C_{18:1}$ on chloroplast lipids (4). It was backcrossed to the wild type three times before being used for the experiments described here. Unless otherwise indicated, plants were grown at 22°C under continuous fluorescent illumination (100–150 $\mu E\ m^{-2}\ s^{-1}$) on a perlite:vermiculite:sphagnum mixture (1:1:1) irrigated with mineral nutrients.

Measurements of Growth Rate

Plants were germinated at 22°C and grown under conditions described above. After 14 d the temperature was adjusted as noted in the text. Samples of four plants were harvested at 3 d intervals, and the fr wt of the aerial portions were measured. The relative growth rate (ω^{-1}) was determined as the slope of the natural logarithm of the average fr wt (in mg) plotted against days since the temperature adjustment.

Extraction and Analysis of Chl, Proteins, and Lipids

Leaves were harvested at the rosette stage (3 weeks) and fr wt was determined. Leaves were ground in 80% acetone to determine Chl concentration. A leaf protein extract was made by homogenizing leaves in cold 20 mM Tricine (pH 8.4), 5 mM $MgCl_2$, and 2.5 mM EDTA. Aliquots of the extract were used for protein determination using a modified Lowry assay (16). Fatty acid composition of total leaf lipids was determined after preparation of fatty acid methyl esters as described (17). Lipids were quantified by gas chromatography using 14:0-methyl ester as an internal standard. Thylakoid membranes were isolated as described below without the addition of BSA to the buffers. Aliquots of thylakoid membranes containing 1 mg mL^{-1} Chl were used for protein and lipid determination.

Isolation of Thylakoid Membranes

Leaves were harvested at the rosette stage and chilled in an ice bath for 5 min. Thylakoid membranes were isolated by grinding leaves in 20 mM Tricine (pH 8.4), 10 mM NaCl, 10 mM EDTA, 450 mM sorbitol, and 0.1% (w/v) BSA. The homogenate was passed through Miracloth (Calbiochem, La Jolla, CA) and centrifuged at 3000*g* for 5 min. The pellet was washed with cold 10 mM Hepes (pH 7.8), 10 mM NaCl, 5 mM EDTA, and dispersed in resuspension buffer containing: 20 mM Hepes (pH 7.8), 10 mM NaCl, 100 mM sorbitol, 2 mM $MgCl_2$, and 2.5 mM EDTA, and 0.1% (w/v) BSA. $MgCl_2$ was omitted from the resuspension buffer for fluorescence measurements as described in the text.

Electrophoresis of Chl-Protein Complexes

Following the procedures of Dunahay and Staehelin (7), 0.2 mg of isolated membranes were suspended in Tris-maleate buffer with 0.88% (w/v) octyl-β-D-glucopyranoside, 0.22% SDS, and 10% glycerol except that the final octyl-glucoside:SDS:Chl ratio was adjusted to 10:2.5:1. Solubilized membranes were stirred on ice for 5 min and centrifuged at 10,000*g* for 5 min to remove starch and debris. Pigment-protein electrophoresis was performed in darkness as described (14), except that 3 mm 10% polyacrylamide gels were used.

Two-Dimensional Gel Electrophoresis

L-[^{35}S]Methionine (1083 Ci $mmol^{-1}$) was diluted to 0.5 mCi mL^{-1} with 0.025% Triton X-100 and applied onto both leaf surfaces of 15 d old plants. Twenty-four h after application of the label, aerial portions of five plants were harvested and thylakoid membranes were isolated as described above. Membrane proteins were extracted and resolved by two-dimensional electrophoresis as described (13).

Photosynthetic Electron Transport Measurements

Whole chain and PSI-dependent electron transport activities were assayed at 25°C in the presence of 0.1 mM NaN_3, using water and 0.5 mM *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (reduced with 2.5 mM ascorbate) as donors, respectively, by monitoring the O_2 consumption by 0.1 mM methyl viologen in a Rank oxygen electrode. Thylakoid membranes (20–30 μg Chl) were added to the reaction mixture which contained 300 mM sorbitol, 20 mM Hepes (pH 7.8), 10 mM NaCl, 2 mM $MgCl_2$, 2.5 mM EDTA, 0.1% BSA, 0.1 μM gramicidin D, and 1 mM NH_4Cl. The PSI assay also contained 1 μM DCMU to inhibit PSII activity and 10 μg mL^{-1} superoxide dismutase. Saturating white light illumination (1200 $\mu E\ m^{-2}\ s^{-1}$) was provided by a high intensity microscope lamp. PSII-mediated 2,6-dichlorophenolindophenol reduction was measured at 580 nm using a Hitachi 100-60 spectrophotometer as described (14).

Low Temperature Chl Fluorescence

Aliquots of thylakoid membranes in resuspension buffer lacking $MgCl_2$ were diluted to a concentration of 10 μg Chl mL^{-1} in 60% (v/v) glycerol, and sodium fluorescein was added as an internal standard to a final concentration of 2 mM (14). Samples were then frozen in liquid N_2 in capillary tubes (0.5 mm i.d.). Fluorescence emission spectra were acquired using an SLM 4048 scanning spectrofluorometer. Excitation was provided by light at 440 nm with a half-bandwidth of 4 nm. Fluorescence emission was scanned in 1 nm increments from 470 to 800 nm with a half-bandwidth of 1 nm. Fluorescence was measured in the presence and absence of 5 mM $MgCl_2$. Acquisition, storage, and mathematical manipulations of spectra were performed by an on-line Hewlett-Packard 9825 computer.

Room Temperature Chl Fluorescence

Room-temperature, fluorescence-induction transients of isolated thylakoid membranes were measured in the presence of 10 μM DCMU. Membranes were diluted in resuspension buffer to a final concentration of 5 μg Chl mL^{-1} and dark-adapted for 5 min before use. The actinic light was provided by a microscope illuminator through a broadband blue optical filter (Corning 4-96), with onset of illumination controlled by an electronic shutter (Vincent Associates, Rochester, NY). Fluorescence was measured through a Corning 2-64 red filter by a photodiode placed 90° to the incident light as described (20). Transients were recorded on a Nicolet Explorer II digital oscilloscope.

Electron Microscopy

Preparation and examination of leaf samples by electron microscopy was carried out as described (16). Quantitative meassurements of membrane profiles on electron micrographs were made on sections of 20 chloroplasts from both wild type and mutant lines. Granal, stromal, appressed, and nonappressed membrane assignments were made as described in Figure 1.

Measurement of Chloroplast Copy Number

Chloroplast copy number per cell was determined in isolated protoplasts as described (17). Aliquots (10–20 μL) of protoplast suspension were pipetted on microscope slides and protoplasts were flattened by the coverslip application, so that the chloroplasts formed a monolayer within cells and could be easily counted.

Effects of Temperature on Chl Fluorescence

Temperature-induced fluorescence yield enhancement was measured on dark-adapted, whole, detached leaves by minor modifications of the method of Schreiber and Berry (26). Weak (0.3 $\mu E\ m^{-2}s^{-1}$) monochromatic light at 480 nm with 4nm half-bandwidth was directed at a 45° angle to a leaf placed between two sheets of 0.1 mm thick Mylar in a water filled cuvette in the SLM spectrofluorometer. Fluorescence emission from the leaf surface was monitored at 700 nm with 2 nm half-bandwidth. The temperature of the sample was increased at a rate of 1°C min^{-1}, and the fluorescence intensity was recorded simultaneously.

RESULTS

Growth of Mutant and Wild-Type *Arabidopsis*

To determine whether the altered fatty acid composition of the *fadC* mutant had an effect on its growth properties, the lipid composition and growth rates of mutant and wild-type plants were examined at a range of temperatures from 10 to 34°C. The lipid analysis of mutant plants indicated that the *fadC* mutation is expressed at all temperatures (data not shown). Both the mutant and wild type had maximal growth rates at approximately 25°C (Fig. 2). However, the growth rate of the mutant was lower than that of the wild type at all temperatures used. The average decrease in the growth rate of the mutant was 14.3%. In contrast, the previously characterized lipid mutants of *Arabidopsis* did not display a reduction in growth rate relative to wild type plants (14, 17). In view of the fact that the mutant line had been backcrossed three times to the wild type, it seems possible that the reduced growth rate is due to the effects of the *fadC* mutation. However, additional generations of backcrossing or the isolation of additional independent alleles of *fadC* will be required to exclude the possibility that another mutation is responsible for the reduced growth rate.

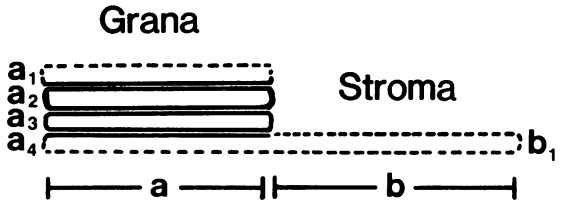

Figure 1. Schematic illustration of chloroplast membrane ultrastructure. Morphometric analysis was performed in accordance with the following rules: (——), appressed membrane; (– – –), nonappressed membrane; a, granal width; b, stromal length; a_1–a_4, granal thylakoids; b_1, stromal thylakoid.

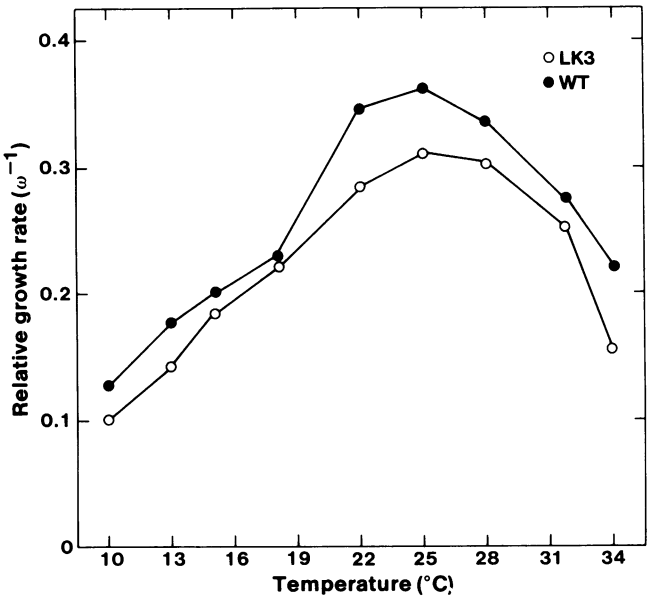

Figure 2. Effect of temperature on the relative growth rate of wild-type and mutant *Arabidopsis*.

Chl, Protein, and Lipid Content of Mutant and Wild-Type *Arabidopsis*

The primary effect of the *fadC* mutation is on the chloroplast pathway of lipid biosynthesis (4). One result of this mutation is a 49% decrease in the degree of polyunsaturation of the major thylakoid lipids. The effects of this change on the amounts of Chl, protein, and lipid were determined on both a whole leaf basis and for isolated thylakoid membranes.

Under standard conditions, the mutant exhibited a slight chlorotic phenotype due to an 18% reduction in total Chl per unit fresh weight (Table I). From the Chl content and Chl *a*/*b* ratio, it can be calculated that the mutant had a 25% reduction in Chl *b* and a 16% reduction in Chl *a*. To determine if the reduction in Chl was related to the *fadC* mutation, 39 F_2 plants from a cross of wild type × LK3 were tested for cosegregation of the *fadC* mutation and decreased Chl content. Of the eight plants that accumulated high levels of $C_{16:1}$ and $C_{18:1}$, all had approximately 15% less Chl than wild type and Chl *a*/*b* ratios above 3.2. The other 31 plants had normal levels of fatty acids and normal Chl content. The cosegregation of the altered lipid composition and the reduction in whole leaf Chl content suggests that these phenotypes are due to the same mutation.

From the change in Chl *a*/*b* ratio it appears that there is a loss of approximately 1.8 molecules of Chl *a* for each molecule

Table I. *Relative Amounts of Chl, Protein, and Lipid in Mutant and Wild-Type Arabidopsis Leaves and Chloroplast Membranes*

The values presented are means ± SD ($n = 3$). *t*-Test was used to determine significantly different values at $P < 0.05$.

Measurement	WT	LK3
Leaves		
Chl/fr wt (mg/g)	1.56 ± 0.03	1.27 ± 0.05*
Chl *a*/*b*	2.91 ± 0.06	3.28 ± 0.07*
Protein/Chl (g/g)	17.78 ± 2.03	19.93 ± 1.76
Lipid/Chl (g/g)	2.55 ± 0.07	2.56 ± 0.01
Protein/lipid (g/g)	6.87 ± 0.78	7.78 ± 0.68
Chloroplast membranes		
Lipid/Chl (g/g)	1.84 ± 0.12	2.29 ± 0.15*
Protein/Chl (g/g)	7.75 ± 0.78	7.30 ± 0.86
Protein/lipid (g/g)	4.21 ± 0.34	3.18 ± 0.47*

* Significantly different from wild type.

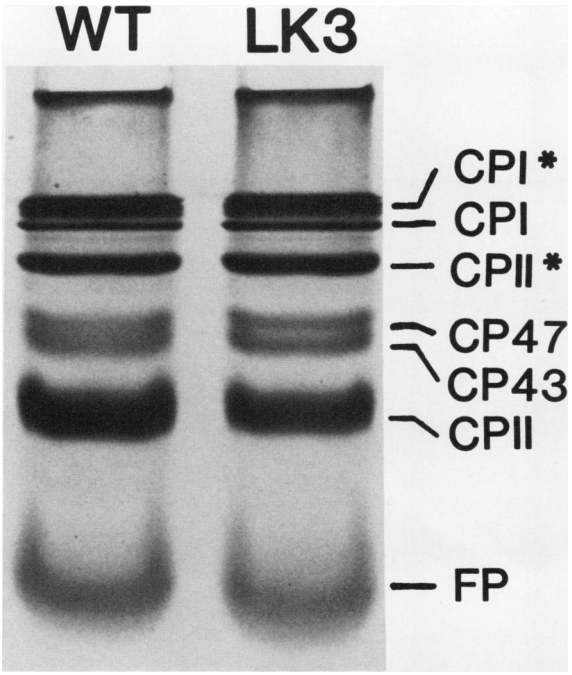

Figure 3. Chl-protein complexes of chloroplast membranes from wild-type and mutant *Arabidopsis*.

Chl *b* lost. All of the Chl in higher plants is believed to be associated with thylakoid proteins (15), therefore the loss of Chl *a* and Chl *b* suggest a reduction in the amount of one or more Chl-protein complexes. Since Chl *b* is bound to LHCP in about a 1:1 stoichometry with Chl *a* (31), an approximately 25% reduction of LHCP would be expected in the mutant. The additional loss of Chl *a* could result from further reductions in the levels of PSI and PSII complexes in the mutant.

To compare directly the levels of LHCP, PSI, and PSII Chl-protein complexes in the mutant and wild type, solubilized Chl-protein complexes were separated by mildly dissociating SDS-polyacrylamide gel electrophoresis (Fig. 3). The identity of the Chl-containing bands resolved by this method (CPI*, CPI, CPII*, CP47, CP43, CPII, and free pigments) was established by comparing the absorption spectra of individual bands with published values (9). Measurement of the amount of Chl in each of the Chl-protein complexes revealed that the mutant contained about 22% less CPII, the LHCP monomer. There was not a measurable reduction in the amount of CPII or CPII*. These results indicate that the reduction in Chl *a* and Chl *b* in the mutant is due in part to a decrease in the relative level of LHCP. No other changes in Chl-protein complexes were apparent.

LHCP is one of the most abundant thylakoid membrane proteins and constitutes about half of the membrane protein in higher plant chloroplasts (31). Therefore, the decline in LHCP levels would be consistent with the loss of about 8% of total thylakoid protein. However, the thylakoid membranes of the mutant exhibited a 25% reduction in protein to lipid ratio. To examine whether this reflected preferential loss of certain proteins, the total polypeptide composition of thylakoid membranes isolated from mutant and wild type after labeling with L-[^{35}S]methionine were compared by two-dimensional SDS-PAGE (data not shown). No major differences were observed between the two genotypes and no missing or additional polypeptides were observed. The fact that changes in the level of LHCP were not observed in this experiment reflects the lack of sensitivity of this technique to small quantitative differences in protein content.

Electron Transport

The effect of decreased lipid unsaturation on photosynthetic electron transport was assayed in thylakoids isolated from mutant and wild type grown under standard conditions (Table II). When expressed on a Chl basis, the light-saturated rates of whole chain, PSI and PSII activities were higher in the mutant than wild type. The apparent increases in electron transport rates are an indication of a greater reduction in the amount of antenna Chl than in Chl associated with the reaction centers. A decrease in the PSII/PSI ratio in the mutant, and the decrease in PSII activity per unit of thylakoid lipid (calculated from Tables I and II), indicate that PSII activity is reduced in the mutant. The amount of PSI activity per unit of thylakoid lipid was not affected by the mutation.

Fluorescence Spectra

At low temperatures (77 K), isolated thylakoid membranes have a characteristic fluorescence emission spectrum that can be resolved into three major peaks at 685, 695, and 734 nm (19). These peaks have been attributed to LHCP associated with PSII, PSII reaction centers, and PSI, respectively. Changes in the peak intensities indicate differences in the efficiency of energy distribution between the Chl-containing

Table II. *Photosynthetic Electron Transport Rates by Chloroplast Membranes from Mutant and Wild-Type Arabidopsis*

Values are means ± SD ($n = 5$).

Activity	WT	LK3	LK3/WT*
	$\mu mol\ O_2\ g\ fr\ wt^{-1}\ h^{-1}$		
PSI + PSII	179 ± 20	192 ± 30	1.07
PSI	320 ± 14	410 ± 18	1.28
PSII	381 ± 14	401 ± 26	1.05

components of the photosynthetic membranes. The 77 K fluorescence spectra of isolated thylakoid membranes of mutant and wild type were determined in order to assess the effect of the lipid changes on distribution of excitation energy between the Chl-protein complexes. In the mutant, the F_{685}/F_{734} ratio was higher, both in the presence and absence of $MgCl_2$ (Table III). $MgCl_2$ regulates a cation-dependent reorganization of protein complexes in isolated thylakoids that causes an increase in energy distribution toward PSII (19). By normalizing the fluorescence emission spectra with an internal fluorescein standard, it was clear that the higher F_{685}/F_{734} ratio was due to a lower absolute fluorescence emission at F_{734} and a concomitant absolute increase in fluorescence at F_{685} (Fig. 4). These changes are indicative of a lower efficiency of excitation energy transfer from LHCP to PSI or of some structural impairment of the peripheral antenna of PSI in the mutant.

Room-temperature Chl fluorescence primarily represents fluorescence emitted from PSII (20). In the presence of DCMU, Chl fluorescence depends only on the exciting light intensity, the number of Chl molecules active in transferring excitation energy to PSII reaction centers, and the efficiency of transfer. The measured F_o value, the initial fluorescence, was similar for thylakoid membranes from mutant and wild type. However, the maximal fluorescence (F_m) and the proportion of Chl active in photochemistry (F_v/F_o), were 17% lower in the mutant (Table III).

Table III. *Low Temperature (77 K) Fluorescence and Room Temperature Fluorescence Induction of Isolated Thylakoids*

Mean values are expressed in arbitrary units ± SD ($n > 5$). *t*-Test was used to determine significantly different values at $P < 0.05$.

Fluorescence Temperature	WT	LK3
77 K fluorescence		
F_{685}/F_{734} $+Mg^{2+}$	0.87 ± 0.06	0.97 ± 0.01*
F_{685}/F_{734} $-Mg^{2+}$	0.65 ± 0.02	0.81 ± 0.02*
Room temperature fluorescence		
F_o	1000 ± 40.5	1027 ± 58
F_m	2720 ± 159	2495 ± 137*
F_v/F_o	1.72 ± 0.07	1.43 ± 0.05*

* Significantly different from wild type.

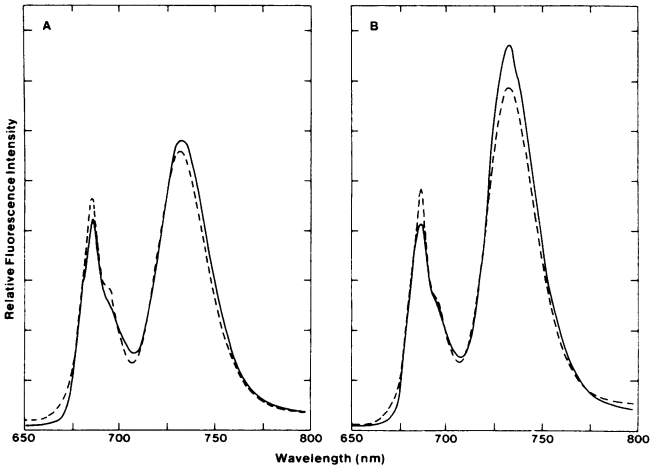

Figure 4. Chl fluorescence spectra of chloroplast membranes from wild-type (——) and mutant (– – –) *Arabidopsis* in the presence (A) and absence (B) of 5 mM $MgCl_2$.

Chloroplast Ultrastructure

Striking changes in chloroplast ultrastructure were observed in the *fadC* mutant. The major effect of this mutation was on the degree of membrane appression (Fig. 5). Morphometric analysis of chloroplasts from mutant and wild type indicated that the average amount of appressed membrane per plastid decreased from 114.1 μm in the wild type to 59.0 μm in the mutant, resulting in a net decline of 48% (Table IV). This was due to a 32% reduction in the number of thylakoids per granum and a 35% reduction in the granal width. The smaller granal size was partially compensated for by an increase in the number of grana per plastid. In contrast with the effects of the *fadC* mutation on granal membrane length, the amount of stromal membrane was normal in the mutant. This resulted

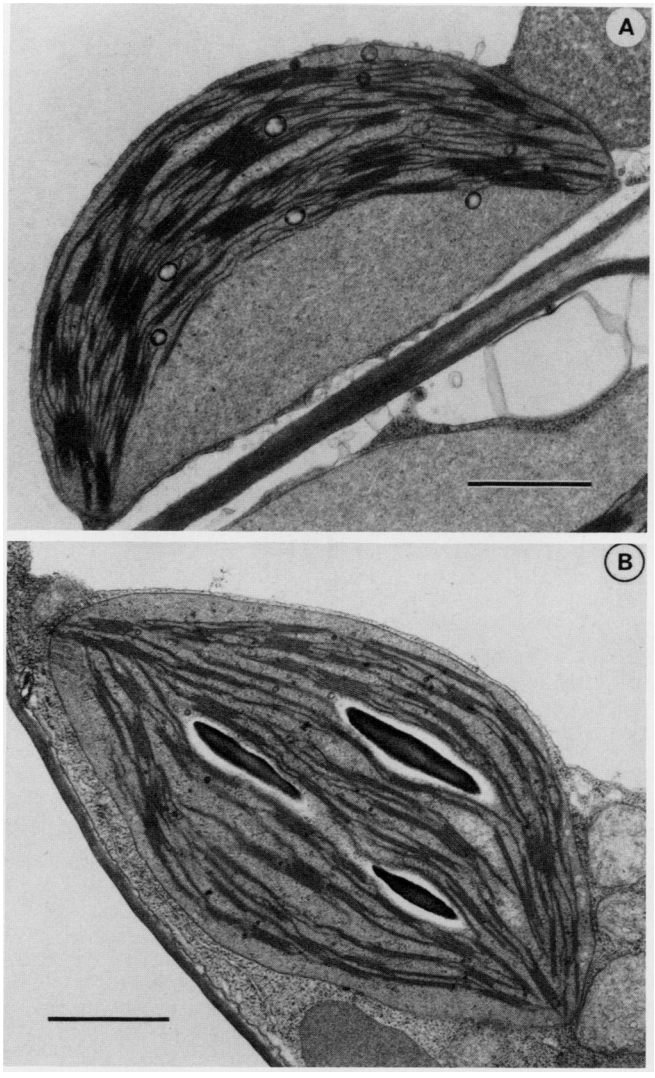

Figure 5. Transmission electron micrographs of chloroplasts from wild-type and mutant rosette leaves of *Arabidopsis*. a, wild type; b, LK3. Bar = 1 μm.

Table IV. *Morphometric Analysis of Chloroplasts from LK3 and Wild-Type Arabidopsis*

Values with SD are means (n = 20). t-Test was used to determine significantly different values at $P < 0.05$. Values without SD are derived from the measurements.

Measurement	WT	LK3
Grana/plastid	54.3 ± 6.6	71.6 ± 6.6*
Thylakoids/granum	6.2 ± 3.7	4.2 ± 2.3*
Granal width (μm)	0.40 ± 0.04	0.26 ± 0.02*
Stroma thylakoid length (μm)	0.18 ± 0.01	0.19 ± 0.01
Stroma thylakoids/plastid	103.9 ± 11.5	110.0 ± 5.6*
Appressed thylakoids/plastid (μm)	114.1	59.0
Nonappressed thylakoids/plastid (μm)	40.7	38.9
Total thylakoids (μm)	154.8	97.9
Appressed/nonappressed thylakoids	2.8	1.5
Surface area (μm^2/plastid)	9.9 ± 1.6	10.0 ± 0.7
Plastids/cell	54.5 ± 19.9	52.2 ± 14.5

* Significantly different from wild type.

in an appressed to nonappressed membrane ratio of 1.5:1 in the mutant compared to a ratio of 2.8:1 in the wild type.

One result of the reduction in appressed membrane in the *fadC* mutant was an overall loss of 36% of the total membrane length on a chloroplast basis. Since there were no changes in the number of chloroplasts per cell, the lower thylakoid membrane length reflects a net decline in the amount of photosynthetic membrane at the whole cell level. This loss of photosynthetic membrane is consistent with the 19% reduction in the leaf lipid content on a fr wt basis in the mutant (Table I).

Effect of High Temperature on Thylakoid Membranes

One measure of the thermal stability of chloroplast membranes is based on the effect of high temperature on Chl fluorescence. A sharp rise in Chl fluorescence occurs upon heating thylakoid membranes. This is thought to be due to the dissociation of LHCP from PSII which results in the emission of energy absorbed by LHCP as fluorescence (26). The temperature at which this occurs provides an index of the thermal stability of photosynthetic membranes (21, 26).

The effect of temperature of Chl fluorescence on intact leaves of mutant and wild type was measured by heating detached leaves at a rate of 1°C min^{-1} from 26°C up to 58°C and continuously monitoring fluorescence. The fluorescence enhancement point of the wild type leaves was about 42.5°C whereas the fluorescence enhancement point of the mutant was 45.5°C (Fig. 6). The difference in the threshold temperatures suggests that the *fadC* mutation results in increased thermal stability of *Arabidopsis* photosynthetic membranes.

Heating of isolated chloroplasts also leads to the inactivation of photosynthetic electron transport (2). This inactivation is probably due to the sensitivity of the water-splitting apparatus of PSII to heat (2). The effect of temperature on whole chain electron transport rates in isolated thylakoid membranes was measured by incubating isolated membranes in

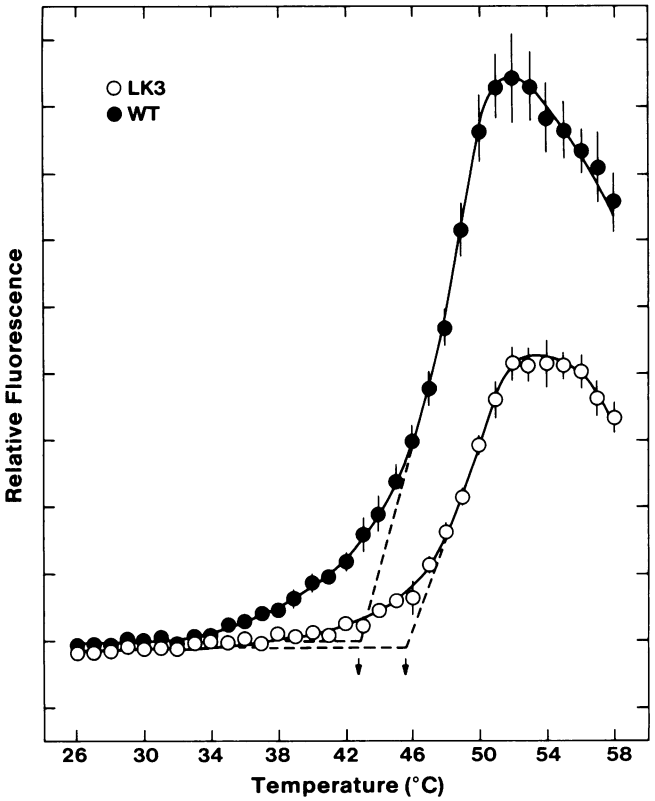

Figure 6. Temperature-induced fluorescence yield enhancement of wild-type and mutant leaves. Plants were grown at 22°C. The arrows indicate estimates of threshold temperatures at which fluorescence was enhanced. Each point represents the mean ± SD (n = 4).

darkness for 10 min at various temperatures from 25 to 45°C, and then measuring whole chain electron transport at 25°C. In both mutant and wild type, increasing preincubation temperatures resulted in progressive inactivation of electron transport rates (Fig. 7). However, the degree of inactivation was significantly higher in the wild type at preincubation temperatures above 30°C. The kinetics of thermal inactivation of photosynthetic electron transport was also examined by incubating isolated chloroplast membranes at 45°C for various times, and then assaying the whole chain activity at 25°C (Fig. 8). Under these conditions the membranes of the mutant are more resistant to thermal inactivation than wild type. Thus, these studies indicate that the degree of unsaturation of the thylakoid lipid matrix can influence the thermal tolerance of photosynthetic membranes in *Arabidopsis*.

DISCUSSION

Lipid Unstaturation and Chloroplast Ultrastructure

Because the *fadC* mutation primarily affects the lipid composition of chloroplast membranes, our analysis of the physiological consequences of the mutation have focused on the structure and function of the chloroplasts. The altered ultrastructure of the chloroplast membranes from the mutant suggests that lipid polyunsaturation is an important factor regulating chloroplast structure. The question which arises concerns the mechanisms by which the altered lipid compo-

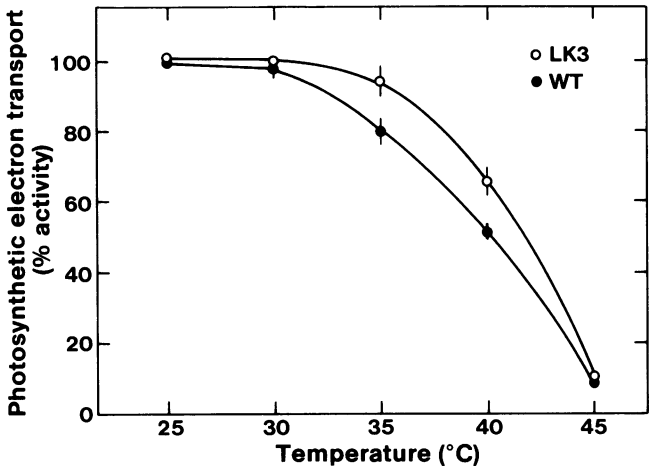

Figure 7. Effect of temperature on photosynthetic electron transport in chloroplast membranes from wild-type and mutant *Arabidopsis*. Activity is expressed relative to that obtained with membranes preincubated in darkness at 4°C for 10 min. Each point represents the mean ± SD (n = 3).

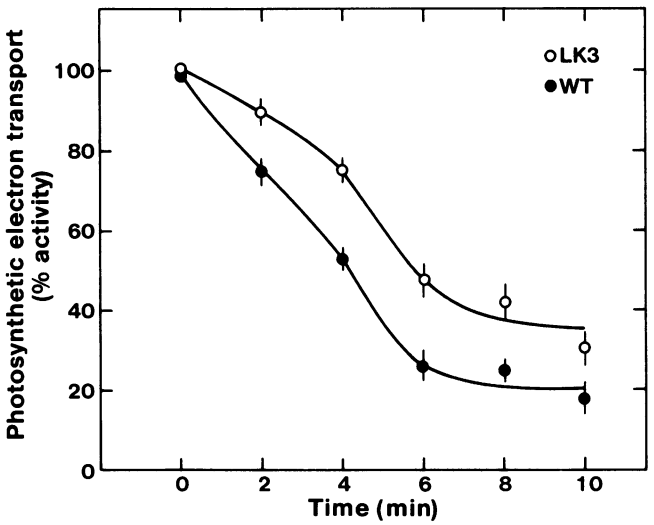

Figure 8. Photosynthetic electron transport activity in chloroplast membranes from wild-type and mutant *Arabidopsis* preincubated at various temepratures for 10 min then assayed at 22°C. Activity is expressed relative to that obtained with membranes preincubated in darkness at 4°C for 10 min. Each point represents the mean ± SD (n = 3).

sition of the mutant brings about the observed changes. There seem to be several nonexclusive possible explanations. First, it is possible that because decreased unsaturation changes the geometry of the lipids, there is a direct effect on the topology of the membranes which is reflected in the altered ultrastructure. In support of this general concept it should be noted that changes in erythrocyte morphology have been induced by changing the lipid composition of the outer leaflet of the plasma membrane (5). However, because large decreases in chloroplast lipid unsaturation resulting from catalytic hydrogenation do not affect chloroplast ultrastructure (25), we consider a direct effect of lipid composition on ultrastructure unlikely.

Another possible explanation for the observed change in the mutant is that the altered lipid composition differentially alters the stability of certain thylakoid proteins which have roles in determining chloroplast ultrastructure. Because the degree of unsaturation may affect the extent to which intrinsic membrane proteins are hydrated and, therefore, the degree to which they interact with each other and with soluble proteins, it is not difficult to envision mechanisms which could bring this about. Thus, the observed decrease in the amount of LHCP and the attendant loss of Chl may reflect the degree to which the turnover of this protein complex is sensitive to the lipid environment. In this respect, it should be noted that a correlation between reduced grana formation and low LHCP levels has been observed in Chl *b*-deficient mutants (3), and it has been proposed that membrane stacking in higher plant chloroplasts is mediated by adhesion between molecules of LHCP (29).

A third possibility is that the changes in ultrastructure reflect a compensatory change that largely obviates the otherwise deleterious effects of the altered lipid composition on photosynthetic electron transport. The differences in ultrastructure between the mutant and wild type are reminiscent of the differences between sun and shade plants, respectively (18). In the case of 'sun plants,' the low proportion of far red illumination is thought to decrease the activity of PSI relative to PSII. By decreasing the amount of stacking, the relative proportions of PSI and PSII activity are brought back into balance, although the net effect of this adaptive process can result in a range of PSI/PSII ratios depending on the species examined (1). By analogy, it is possible that in some way the altered lipid composition in the mutant triggers the same mechanisms which lead to the change in ultrastructure associated with the sun/shade adaptation.

In contrast with the *fadC* mutant, the *fadD* mutant of *Arabidopsis* does not exhibit a reduced percentage of granal membrane, but contains a greater number of smaller chloroplasts per cell (17). While both of these mutants are defective in the desaturation of a similar class of chloroplast lipids, they differ in their acyl chain specificity. The *fadC* mutant is defective in *cis* *n*-6 desaturation of monounsaturated lipids while *fadD* mutants are defective in *cis* *n*-3 desaturation of diunsaturated lipids (4, 17). Since these two mutations cause reduced chloroplast lipid unsaturation but affect different aspects of chloroplast ultrastructure, we conclude that changes in the acyl chain composition of chloroplast lipids may influence chloroplast ultrastructure in a highly specific manner.

Chloroplast Function

A deleterious effect of the *fadC* mutation on PSII activity is partially obscured by the apparent increase in activity per unit Chl. However, the 20% decrease in PSII/PSI activity and the 20% decrease in PSII activity per unit of thylakoid lipid both suggest a specific reduction of PSII activity. A reduction of similar magnitude in whole-chain electron transport activity per unit lipid can be accounted for by the reduction in PSII activity. Similar effects of lipid saturation on electron transport rates were also observed following *in vitro* catalytic

hydrogenation of thylakoid membranes (12, 25, 32). Progressive saturation of double bonds of lipids inhibited whole chain and PSII electron transport rates by up to 15 and 33%, respectively (32). Thus, our results corroborate the conclusion reached in catalytic hydrogenation experiments, that lipid unsaturation may function in maintaining membrane properties required for normal electron transport rates. Additional studies will be required to pinpoint the basis for this effect of polyunsaturation on PSII activity.

There may be more than one effect of polyunsaturation on photosynthetic electron transport. Horvath *et al.* (12) have shown using catalytic hydrogenation, that high levels of thylakoid lipid saturation cause relatively increased PSII fluorescence due to the dissociation of peripheral-LHCP from PSII. The increase in 77 K fluorescence at 685 nm (corresponding to LHCP) in the *fadC* mutant is consistent with the concept that a decrease in lipid unsaturation may cause partial dissociation of LHCP from PSII. A similar conclusion was reached in thylakoid membrane reconstitution experiments (27) in which reconstitution of energy transfer from LHCP to the reaction centers in detergent solubilized thylakoid membranes required highly unsaturated MGD, suggesting that lipid unsaturation is important for the assembly of LHCP-reaction center complexes. It seems likely that this apparent requirement is a nonspecific effect which is a common characteristic of many thylakoid membrane proteins.

Enhanced Thermal Tolerance

Many species of desert plants exhibit physiological adaptation to growth at elevated temperatures which results in enhanced thermal tolerance of the photosynthetic apparatus (2). The increased thermal stability, measured as the temperature at which steady state fluorescence was enhanced, varied from 5 to 7°C for winter annuals, and 3 to 4°C for summer annuals (6). The biochemical basis for this effect is not known. However, several authors have observed that a decrease in the degree of lipid unsaturation is associated with the acclimation phenomenon (22, 24). This correlation has raised the possibility that the degree of lipid unsaturation is a factor in the acclimation response. In a direct test of this hypothesis, Thomas *et al.* (30) employed catalytic hydrogenation of pea thylakoids to alter the degree of lipid unsaturation. Comparisons of the effect of temperature on Chl fluorescence and freeze-fracture studies of hydrogenated and control membranes were consistent with the concept that lipid unsaturation plays a direct role in thermal stability of the membranes. These authors suggested that decreased lipid unsaturation raises the temperature at which non bilayer forming lipids such as MGD phase-separate into nonbilayer structures which disrupt thylakoid organization.

The photosynthetic membranes of the *fadC* mutant showed superior thermal stability by two criteria: a 3 to 4°C increase in the temperature at which fluorescence yield was enhanced, and a slower rate of inactivation of electron transport at high temperatures. The difference in thermal tolerance of the chloroplast membranes of the *fadC* mutant, compared to the wild type, is comparable in degree to that observed in natural populations of thermally adapted and nonadapted plants (6). The fact that this enhanced thermal tolerance can be induced by a mutation which specifically reduces the amount of desaturation of chloroplast lipids provides strong support for the concept that lipid unsaturation is a determinative factor in this adaptive response. Our results are consistent with the hypothesis that it is the reduced tendency of less unsaturated membranes to form nonbilayer structures which is responsible for the enhanced thermal stability (30).

ACKNOWLEDGMENT

The excellent technical assistance of Linda Savage is gratefully acknowledged.

LITERATURE CITED

1. **Anderson JM, Chow WS, Goodchild DJ** (1988) Thylakoid membrane organization in sun/shade acclimation. Aust J Plant Physiol **15:** 11–26
2. **Berry J, Bjorkman O** (1980) Photosynthetic response and adaptation to temperature in higher plants. Annu Rev Plant Physiol **31:** 491–543
3. **Bolton P, Warfe J, Harwood JL** (1987) The lipid composition of a barley mutant lacking chlorophyll b. Biochem J **174:** 67–72
4. **Browse J, Kunst L, Anderson S, Hugly S, Somerville CR** (1989) A mutant of *Arabidopsis* deficient in the chloroplast 16:1/18:1 desaturase. Plant Physiol **90:** 522–529
5. **Christiansson A, Kuypers FA, Roelofsen B, Op Den Kamp JAF, Van Deenen LLM** (1985) Lipid molecular shape affects erythrocyte morphology. J Cell Biol **101:** 1455–1462
6. **Downton WJS, Berry JA, Seemann JR** (1984) Tolerance of photosynthesis to high temperature in desert plants. Plant Physiol **74:** 786–790
7. **Dunahay TG, Staehelin LA** (1985) Isolation of photosystem I complexes from octyl glucoside/sodium dodecyl sulfate solubilized spinach thylakoids, Plant Physiol **78:** 606–613
8. **Galey J, Francke B, Bahl J** (1980) Ultrastructure and lipid composition of etioplasts in developing dark-grown wheat leaves. Planta **149:** 433–439
9. **Green BR** (1988) The cholorphyll-protein complexes of higher plant photosynthetic membranes or just what green band is that? Photosynth Res **15:** 3–32
10. **Gounaris K, Barber J** (1983) Monogalactosyldiacylglycerol: the most abundant polar lipid in nature. Trends Biochem Sci **8:** 378–381
11. **Harwood JL** (1982) Plant acyl lipids. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 4. Academic Press, New York, pp 1–55
12. **Horvath G, Melis A, Hideg E, Droppa M, Vigh L** (1987) Role of lipids in the organization and function of photosystem II studied by homogenous catalytic hydrogenation of thylakoid membranes in situ. Biochim Biophys Acta **891:** 68–74
13. **Hurkman WJ, Tanaka CK** (1986) Solubilization of plant membrane proteins for analysis by two dimensional gel electrophoresis. Plant Physiol **81:** 802–806
14. **Kunst L, Browse J, Somerville C** (1989) Altered chloroplast structure and function in a mutant of *Arabidopsis* deficient in plastid glycerol-3-phosphate acyltransferase activity. Plant Physiol **90:** 846–853
15. **Markwell JP, Thornber JP, Boggs RT** (1979) Higher plant chloroplasts: evidence that all the chlorophyll exists as chlorophyll-protein complexes. Proc Natl Acad Sci USA **76:** 1233–1235
16. **Markwell MA, Haas SM, Bieber LL, Tolbert NE** (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem **87:** 206–210
17. **McCourt P, Kunst L, Browse J, Somerville CR** (1987) The effects of reduced amounts of lipid unsaturation on chloroplast ultrastructure and photosynthesis in a mutant of *Arabidopsis.* Plant Physiol **84:** 353–360

18. **Melis A** (1984) Light regulation of photosynthetic membrane structure, organization and function. J Cell Biochem **24:** 271–285
19. **Murata N, Satoh K** (1986) Absorption and fluorescence emission by intact cells, chloroplasts, and chlorophyll-protein complexes. *In* Govindjee, J Amesz, DC Fork, eds, Light Emission by Plants and Bacteria. Academic Press, New York, pp 137–159
20. **Paterson DR, Arntzen CJ** (1982) Detection of altered inhibition of photosystem II reactions in herbicide-resistant plants. *In* M Edelman, R Hallick, NH Chua, eds, Methods in Chloroplast Molecular Biology. Elsevier, Amsterdam, pp 109–119
21. **Pearcy RW** (1976) Temperature effects on growth and CO_2 exchange rates of *Atriplex lentiformis.* Oecologia **25:** 245–255
22. **Pearcy RW** (1978) Effect of growth temperature on the fatty acid composition of the leaf lipids in *Atriplex lentiformis* (Torr.) Wats. Plant Physiol **61:** 484–486
23. **Quinn PJ** (1987) Lipid phase behaviour and lipid-protein interactions in the chloroplast photosynthetic membrane. Biochem Soc Trans **15:** 86–91
24. **Raison JK, Roberts JKM, Berry JA** (1982) 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. Biochim Biophys Acta **688:** 218–228
25. **Restall CJ, Williams P, Percival MP, Quinn PJ, Chapman D** (1979) The modulation of membrane fluidity by hydrogenation processes. Biochim Biophys Acta **555:** 119–130
26. **Schreiber U, Berry JA** (1977) Heat induced changes in chlorophyll fluorescence in intact leaves correlated with damage in the photosynthetic apparatus. Planta **136:** 233–238
27. **Siefermann-Harms D, Ross JW, Kaneshiro KH, Yamamoto HY** (1982) Reconstitution by monogalactosyldiacylglycerol of energy transfer from light-harvesting chlorophyll a/b-protein complex to the photosystems in Triton X-100-solubilized thylakoids. FEBS Lett **149:** 191–196
28. **Somerville CR, Browse J** (1988) Genetic manipulation of the fatty acid composition of plant lipids. *In* EE Conn, ed, Opportunities for Phytochemistry in Plant Biotechnology Recent Advances in Phytochemistry, Vol 22. Plenum Press, New York, pp 19–44
29. **Staehelin LA, Arntzen CJ** (1983) Regulation of chloroplast membrane function: protein phosphorylation changes in spatial organization of membrane components. J Cell Biol **97:** 1327–1337
30. **Thomas PG, Dominy PJ, Vigh L, Mansourian AR, Quinn PJH, Williams WP** (1986) Increased thermal stability of pigment-protein complexes of pea thylakoids following catalytic hydrogenation of membrane lipids. Biochim Biophys Acta **849:** 131–140
31. **Thornber PJ** (1987) Biochemical characterization and structure of pigment-proteins of photosynthetic organism. *In* LA Staehelin, CJ Arntzen, eds, Photosynthesis III, Vol 19. Springer-Verlag, New York, pp 96–142
32. **Vigh L, Joo F, Droppa M, Horvath LI, Horvath G** (1985) Modulation of chloroplast membrane lipids by homogenous catalytic hydrogenation. Eur J Biochem **147:** 477–481