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Analysis of Photosynthetic Antenna Function in a Mutant of *Arabidopsis thaliana* (L.) Lacking *trans*-Hexadecenoic Acid[1]

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ABSTRACT

Several lines of evidence support the proposal that the unusual chloroplast-specific lipid acyl group Δ3,*trans*-hexadecenoic acid (*trans*-$C_{16:1}$) stimulates the formation or maintenance of the oligomeric form of the light-harvesting chlorophyll *a/b* complex (LHCP). To assess the functional significance of this apparent association we have analyzed LHCP structure and function in a mutant of *Arabidopsis thaliana* (L.) which lacks *trans*-$C_{16:1}$ by electrophoretic analysis of the protein-chlorophyll complexes and by measurements of chlorophyll fluorescence under a variety of conditions. By these criteria the putative oligomeric form of LHCP appears to be slightly more labile to detergent-mediated dissociation in the mutant. The oligomeric PSI chlorophyll-protein complex, associated with PSI, was also more labile to detergent-mediated dissociation in the mutant, suggesting a previously unsuspected association of *trans*-$C_{16:1}$ with the PSI complex. However, no significant effect of the mutation on the efficiency of energy transfer from LHCP to the photochemical reaction centers was observed under any of the various conditions imposed. Also, the stability of the chlorophyll-protein complexes to temperature-induced dissociation was unaffected in the mutant. The role of *trans*-$C_{16:1}$ is very subtle or is only conditionally expressed.

The chloroplast membranes of all photosynthetic eukaryotes contain the unusual fatty acyl group Δ3, *trans*-$C_{16:1}$[3] which is always found esterified to the second position of phosphatidyl glycerol (7). The fatty acid is atypical because of the *trans* configuration, and because of the position of the double bond near the carboxyl rather than the methyl end of the fatty acid. A specific role for the acyl group in photosynthesis has frequently been proposed because *trans*-$C_{16:1}$-PG occurs only in chloroplast membranes (7), and is present in relatively low amounts in etioplasts but accumulates upon light-induced chloroplast development in parallel with the accumulation of the LHCP and the development of appressed membranes (7, 9, 18). Also, removal of *trans*-$C_{16:1}$ from PG by phospholipase-A2 treatment of isolated thylakoids was reported to alter the efficiency of light capture and to change the kinetics of fluorescence induction (8). However, a specific role in photosynthesis has not been demonstrated (reviewed in [7]).

Recently, evidence pertaining to a possible role for *trans*-$C_{16:1}$ was obtained from experiments in which the lipid content of isolated Chl-protein complexes was characterized. When thylakoid Chl-protein complexes are solubilized in low amounts of SDS and electrophoresed in polyacrylamide gels which also contain low concentrations of SDS, the Chl-protein complexes separate into a characteristic pattern of about six major Chl-containing bands (1). When these bands were extracted from the acrylamide gel and the lipid composition of each band measured, it was found that the LHCP[1] band, which is believed to correspond to an oligomeric form of LHCP, was significantly enriched with *trans*-$C_{16:1}$ (28). The possible importance of the lipid in maintaining the LHCP[1] conformer was also suggested by experiments in which treatment of thylakoids with phospholipase-A2 before solubilization and electrophoresis caused the disappearance of the LHCP oligomer (23). Thus, it has been suggested that *trans*-$C_{16:1}$-PG may be an integral component of the LHCP oligomer, which has been proposed to be the native form of the complex *in vivo* (14). Although it may be only coincidental, there is approximately enough *trans*-$C_{16:1}$ in the chloroplast membranes to satisfy a stochiometry of one molecule per LHCP oligomer (7). A role in LHCP oligomer formation or stabilization is also suggested by recent experiments showing that the rate of reconstitution of LHCP oligomer in liposomes is stimulated by the presence of *trans*-$C_{16:1}$ (24).

We (5) have recently described the isolation of a mutant of *Arabidopsis thaliana* (L.) which specifically lacks *trans*-$C_{16:1}$ and has a compensating increase in palmitic acid (16:0). The mutant is, therefore, believed to lack a specific desaturase which converts palmitic acid at position two of PG to *trans*-$C_{16:1}$. The mutant, which has no obvious phenotype, was isolated by analyzing the fatty acid composition of several thousand randomly selected individuals from a mutagenized population. In a preliminary analysis of thylakoid ultrastructure and function we were unable to establish a difference between the mutant and the wild type. Here, we describe the results of experiments designed to test the role of *trans*-$C_{16:1}$ on formation of LHCP oligomer and on the functional association of LHCP and the photochemical reaction centers. Although fluorescence measurements suggest normal LHCP function, the LHCP oligomer appears less stable to dissociation by SDS in the mutant. A similar effect on the CP1a complex suggests that *trans*-$C_{16:1}$ also stabilizes the presumed oligomeric form of the PSI Chl-protein complex.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The mutant line JB60 was isolated from the Columbia wild type of *Arabidopsis thaliana*

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[3] Abbreviations: *trans*-$C_{16:1}$, *trans*-hexadecenoic acid; LHCP, light-harvesting Chl *a/b* protein complex; CP1, oligomeric PSI Chl-protein complex; PG, phosphatidyl glycerol; LHCP[1], oligomeric form of LHCP; *fadA*, the symbol for a gene which controls the synthesis of *trans*-$C_{16:1}$; LHCP[3], monomeric form of LHCP; F_v, variable fluorescence; F_o, initial fluorescence; F_m, maximum fluorescence.

(L.) Heynh. as previously described (5). The mutant carries a defective allele of a locus, designated *fadA*, which is required for the desaturation of palmitic acid at position two of PG. The mutant has no obvious phenotype and is not visually distinguishable from the wild type. Plants were grown in continuous fluorescent illumination (200 μE m^{-2} s^{-1}) at 24°C and 60% RH on a perlite:vermiculite:sphagnum (1:1:1) mixture irrigated with mineral nutrients (26).

Preparation of Chloroplast Membranes. Thylakoid membranes were isolated by grinding leaves in 50 mM Tricine (pH 7.8), 10 mM NaCl, 10 mM EDTA, and 400 mM sorbitol. The homogenate was passed through four layers of cheesecloth and centrifuged at 3000 *g* for 5 min. The pellet was washed with 10 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM EDTA, and dispersed in resuspension buffer containing: 10 mM Tricine (pH 7.8), 10 mM NaCl, 100 mM sorbitol, and 5 mM $MgCl_2$. In some instances, described in the text, $MgCl_2$ was omitted from the resuspension buffer. Chl concentration was determined in 80% (v/v) acetone (19).

Room Temperature Chlorophyll Fluorescence Measurements. Thylakoids were diluted in resuspension buffer to a final concentration of 5 μg Chl/ml. Illumination was provided by a Unitron microscope illuminator powered by a model C5-6,6A stable output power supply (Power One, Cornello, CA). The actinic light was filtered 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 previously described (21). The voltage output signal was stored on a Nicolet Explorer II digital recording oscilloscope.

Low-Temperature Chlorphyll Fluorescence Measurements. Isolated thylakoids were incubated in resuspension buffer lacking $MgCl_2$ at 4°C for 2 h before use. Aliquots were diluted to a concentration of 10 μg Chl/ml in 60% (w/v) glycerol and then frozen in liquid N_2 in capillary tubes (0.5 mm i.d.). For measurements on intact leaves a wet leaf was placed on a metal spatula so that the leaf was held in place by surface tension. The spatula was then immersed in a cuvette containing liquid N_2 so that the plane of the leaf was at an angle of approximately 45° to the exciting light. Fluorescence emission spectra were recorded using an SLM4048 scanning spectroflurometer (SLM Instruments, Urbana, IL) operating in the ratiometric acquisition mode. Excitation was provided by light at 480 nm with a half-bandwidth of 4 nm. Fluorescence emission was scanned in 0.5 nm increments from 650 to 800 nm with a half-bandwidth of 1 nm. Acquisition, storage, and mathematical manipulation of spectra were performed by an on-line Hewlett-Packard 9825 computer.

Effects of Temperature on Chlorophyll Fluorescence. Fluorescence yield (F_o) was measured on dark-adapted whole detached leaves enclosed in a water-jacketed cuvette similar in design to that described by Schreiber and Berry (25). Weak (0.3 μE m^{-2} s^{-1}) monochromatic light at 480 nm with a 4-nm half-bandwidth was directed at a 45° angle to the leaf which was placed in the spectrofluorometer sample compartment. Fluorescence emission from the leaf surface was monitored at 700 nm with a 2-nm half-bandwidth. Sample temperature was increased at a rate of about 1.5°C min^{-1}. Measurements were made by simultaneously recording leaf temperature and the fluorescence intensity.

Protein Pigment Complexes. Chloroplast membranes were isolated as described above except that for low-salt isolations described in the text, NaCl and $MgCl_2$ were omitted from the isolation and resuspension buffers. Membranes were solubilized before electrophoresis by incubation for 5 min at 22°C in a volume of solubilization buffer (300 mM Tris·Cl [pH 8.8], 10% [v/v] glycerol, and 5% [w/v] SDS) which gave an SDS:Chl ratio of 1:10. In some instances, noted in the text, NaCl was added to the solubilization buffer. The absorbance of the pigment-containing bands in polyacrylamide gels was determined at 600 nm using a Gelman ACD-18 automatic computing densitometer.

RESULTS

Electrophoretic Separation of Chl-Protein Complexes. Previous studies have shown that *trans*-$C_{16:1}$-PG comigrates with the LHCP oligomer in SDS polyacrylamide gels under conditions in which the thylakoid proteins were solubilized with low amounts of SDS so that the Chl-protein associations remain intact (1). It was, therefore, of interest to examine the effect of the *fadA* mutation on the pattern of Chl-protein complexes resolved by this method. Separation of the Chl-protein complexes from wild-type *Arabidopsis* extracts revealed five major Chl-containing bands (Fig. 1). The identity of the major bands as CP1a, CP1, $LHCP^1$, $LHCP^3$, and free Chl was established by comparison of the absorbtion and fluorescence spectra of the bands excised from SDS-polyacrylamide gels with published values (1).

Comparison of the electrophoretic separation patterns of the Chl-protein complexes from the wild-type and the *fadA* mutant under standard conditions revealed that the mutant lacked the two Chl-containing bands designated CP1a and $LHCP^1$ (Fig. 1). These bands are believed to represent the oligomeric forms of CP1 (the P700-Chl *a*-protein complex) and $LHCP^3$ (the presumed LHCP monomer), respectively (1). The absence of $LHCP^1$ in the mutant mimics the similar results obtained following removal of the acyl group at position two of PG by phospholipase-A2 treatment of thylakoids (23). Thus, it appears that by these independent criteria the presence of *trans*-$C_{16:1}$-PG in the chloroplast membranes stabilizes $LHCP^1$ against SDS-mediated dissociation.

The reduction of the amount of CP1a in the extracts of the mutant was unexpected since *trans*-$C_{16:1}$-PG has not previously been reported to be a component of this Chl-protein complex. However, a Chl *a/b* protein complex associated with the PS-I complex has recently been reported (11, 15, 16). The results presented here raise the possibility that this complex has *trans*-$C_{16:1}$-PG specifically associated with it as a boundary lipid. Alternatively, the presence of *trans*-$C_{16:1}$-PG in the membrane may exert a nonspecific effect on both $LHCP^1$ and CP1a stability.

Studies of cation effects on Chl-protein complexes have shown that removal of cations from solubilization buffers increased the proportion of Chl found in CP1a and $LHCP^1$ following electro-

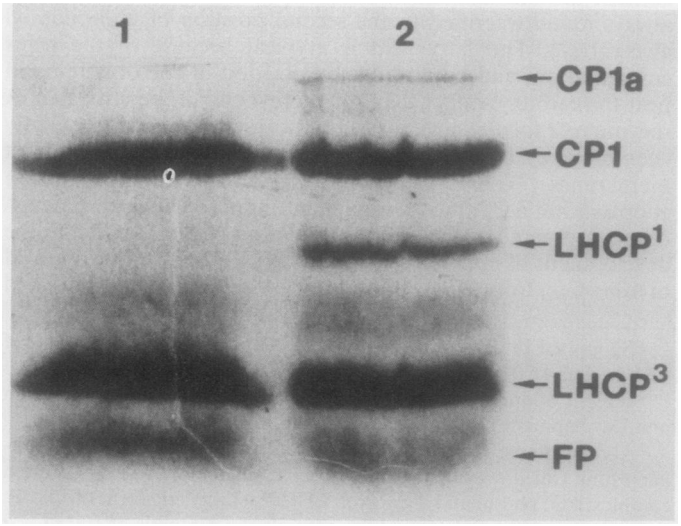

FIG. 1. Pigment protein complexes of mutant (lane 1) and wild-type (lane 2) thylakoids. FP denotes free pigment.

phoresis in SDS polyacrylamide gels (2, 3). The addition of either $MgCl_2$ or NaCl converted an increased proportion of the oligomers into their respective monomers. Therefore, we examined the effect of cation concentration on the proportion of Chl associated with LHCP[1] to determine if conditions could be found which would stabilize the oligomers of the mutant. The solubilization of thylakoid membranes in solutions of SDS containing very low concentrations of NaCl revealed that the thylakoids of the *fadA* mutant contained normal levels of the LHCP[1] oligomer (Fig. 2). As the NaCl concentration was increased from 0 to 100 mM, the amount of LHCP[1] in both mutant and wild type decreased from a maximum of about 7% to zero (Fig. 2). However, the membranes from the mutant were much more sensitive to salt-induced dissociation of LHCP oligomer than those of the wild-type. The concentration of NaCl which gave 50% reduction in LHCP[1] concentration was about 13 mM in the mutant as compared to about 37 mM in the wild type (Fig. 2). Thus, it appears that *trans*-$C_{16:1}$ is not required for LHCP[1] formation but in some way stabilizes the oligomer so that it is less suceptible to SDS-mediated dissociation.

Fluorescence Spectra. Chl fluorescence emission spectra are sensitive indicators of the efficiency of energy distribution between the Chl-containing components of the photosynthetic membranes. Preferential excitation of Chl *b* using 480 nm light results in most of the energy being initially absorbed by LHCP from where excitation energy is distributed between the two photosystems. Detachment of LHCP from one or both of the reaction centers results in relatively increased fluorescence emission from LHCP and associated Chl-protein complexes. Thus, it is possible to monitor the relative extent of interactions between LHCP and the reaction centers in thylakoid membranes by comparing the fluorescence emissions at 685, 695, and 735 nm which have been attributed to LHCP, PSII, and PSI, respectively (6, 20).

To test for the presence of an *in vivo* difference between LHCP function in mutant and wild type we first compared the spectrum of Chl fluorescence from whole leaves at 77K (Fig. 3). An alteration in the efficiency of exciton transfer from LHCP to the photosystems in the mutant would have been expected to result in a change in the ratio of LHCP fluorescence (685 nm) relative to the other emission maxima. However, no significant difference was apparent between the mutant and the wild type by this criterion (Fig. 3).

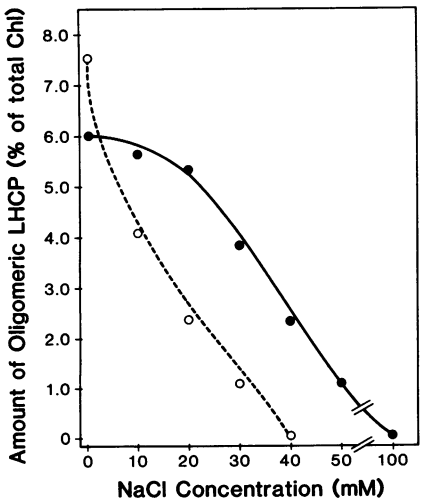

FIG. 2. Effect of NaCl concentration on the proportion of Chl in LHCP oligomer in mutant (O) and wild-type (●) *Arabidopsis*. The percentage of LHCP was determined from a densitometer tracing of the lanes of an SDS-polyacrylamide gel loaded with thylakoid samples which had been incubated in varying concentrations of NaCl.

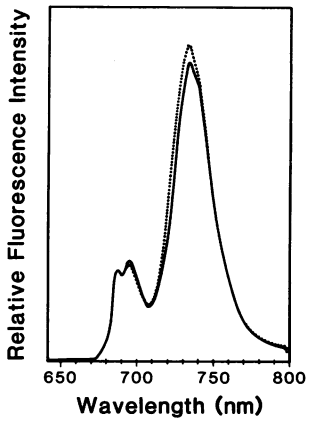

FIG. 3. Chl fluorescnece spectra of whole leaves from mutant (···) and wild-type (——) *Arabidopsis* at 77K.

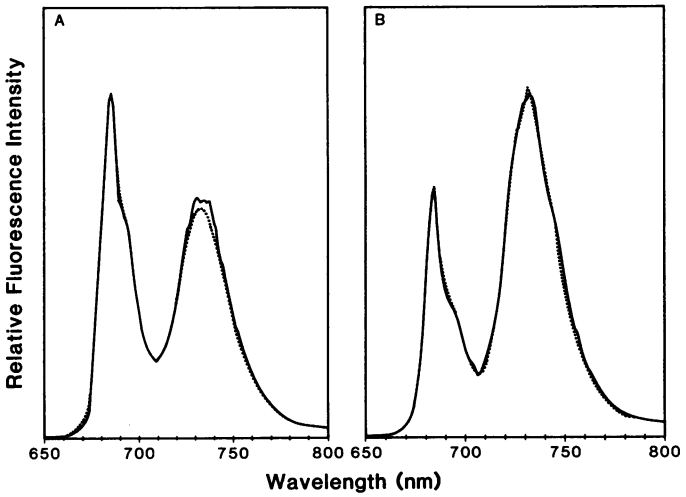

FIG. 4. Chl fluorescence spectra of chloroplasts from mutant (···) and wild-type (——) *Arabidopsis* in the presence (A) and absence (B) of $MgCl_2$ (5 mM). The same preparation of chloroplasts was used for A and B.

In an attempt to relate the effects of cations on LHCP[1] stability to a functional property of LHCP, we examined the effect of cations on low temperature (77K) fluorescence emission spectra of isolated thylakoid membranes. Assuming that the LHCP oligomer is the native form *in situ* (14), it might be expected that if cations induced dissociation of the LHCP oligomer in intact membranes, this would be reflected in less efficient transfer of excitons from LHCP to the reaction centers. In this case one would expect more fluorescence at 685 nm and less at 734 nm. It should be noted, however, that a cation-induced change in the ratio of PSI to PSII fluorescence has previously been attributed to changes in the spatial organization of the Chl-proteins rather than to changes in the quaternary structure of individual proteins (27).

The Chl fluorescence spectrum of chloroplast membranes isolated in the absence of cations is presented in Figure 4B. The spectrum, which is qualitatively very similar to that obtained with whole leaves, was not significantly different with respect to the wavelength of the emission maxima or the relative distribution of fluorescence between the two photosystems in mutant and wild type. Addition of 5 mM $MgCl_2$ to the thylakoids caused an increase in the ratio of PSII to PSI fluorescence (Fig. 4A) as expected from previous studies concerning the effect of divalent cations on membrane appression and fluorescence (6, 27). How-

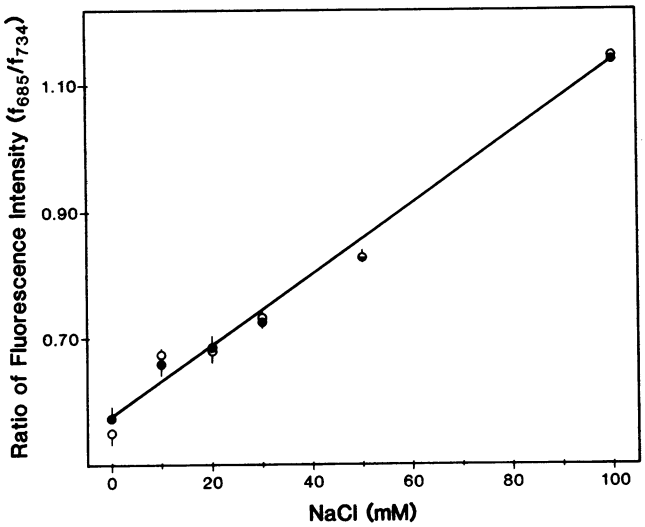

FIG. 5. Ratio of fluorescence intensity from low temperature (77K) emission spectra of the wild type (●) and mutant (○) thylakoids. Each point represents the mean ± SE (n = 3).

Table I. *Room Temperature Fluorescence Induction Parameters of Isolated Thylakoids from Mutant and Wild-Type Arabidopsis in the Presence and Absence of 5 mM $MgCl_2$*

Measurements of F_o, the initial fluorescence, were taken within 2 ms after the shutter opening. F_m, the maximum fluorescence, was measured in the presence of 10 μM DCMU. All samples contained 5 μg Chl *a* + *b*/ml.

Strain	F_o	F_m	F_v/F_o
Wild type (+Mg)	1164 + 114	4746 + 97	3.07 + 0.14
JB60 (+Mg)	1122 + 35	4735 + 225	3.22 + 0.07
Wild type (−Mg)	1027 + 35	2333 + 71	1.27 + 0.02
JB60 (−Mg)	1010 + 13	2302 + 89	1.28 + 0.07

ever, the ratio of PSI to PSII fluorescence in the mutant was not significantly different from that of the wild type.

A more extensive analysis of cation effects was performed by examining the effects of a range of NaCl concentrations of the ratio of PSII to PSI fluorescence (Fig. 5). Addition of NaCl to photosynthetic lamellae caused an essentially linear increase in the ratio of fluorescence at 685 nm to that at 734 nm (Fig. 5). As noted above, a cation stimulated increase in PSII activity at the expense of PSI has previously been observed and is attributed to a decrease in energy spillover from PSII to PSI due to cation-induced changes in the spatial separation of PSI and PSII which decreases the probability of exciton migration from PSII to PSI. In contrast to the results from the SDS-acrylamide gel experiments, no differential cation effect on the transfer of energy from LHCP to PSI in the mutant *versus* the wild type was detected in the range of 0 to 100 mM NaCl (Fig. 5). Energy transfer between LHCP and PSII and between the photosystems does not appear to be impaired as one might expect if LHCP structure was substantially altered (27).

Fluorescence Induction. In previous studies of the role of *trans*-$C_{16:1}$-PG, thylakoid membranes were depleted of *trans*-$C_{16:1}$ by treatment with phospholipase-A2 (8, 22, 23). Membranes treated in this way exhibited altered fluorescence induction kinetics which were interpreted as a reduction in the efficiency of light capture and the rate of plastoquinone reduction (8). To reexamine the relevance of these observations to understanding the role of *trans*-$C_{16:1}$-PG, the function of PSII and LHCP in wild type and the mutant lacking *trans*-$C_{16:1}$-PG was compared by examining the kinetics of induction of room temperature fluorescence at 700 nm. Room temperature fluorescence primarily represents fluorescence emitted from PSII (21). When electron transport is blocked with DCMU, the rise of the variable fluorescence (F_v) is a measure of the time required to close (cause a turnover) all PSII reaction centers (thereby reaching maximal fluorescence, F_m). In this respect, the rise time of F_v is a relative measure of both the number of Chl active in transferring excitation energy to PSII reaction centers and of the efficiency of transfer. The minimum level of fluorescence, F_o, is due to emission from the antenna Chl of PSII which occurs before the excitation energy is trapped by the reaction centers [6].

F_o and the proportion of Chl active in photochemistry (F_v/F_o) appeared to be identical in the mutant and the wild type in the absence of $MgCl_2$ (Table I). Addition of 5 mM $MgCl_2$ to the membranes resulted in a dramatic increase in F_m due to Mg-induced changes in the spatial organization of the membranes (27) and a resulting decrease in the amount of spillover of excitation energy to PSI (6). The effect of cations on fluorescence characteristics of mutant and wild-type membranes was quantitatively and qualitatively indistinguishable under these conditions. These observations, in conjunction with previous studies showing that the mutant and the wild type have indistinguishable rates of electron transport (5), suggest that the two genotypes have indistinguishable PSII photochemistry efficiency. This implies that the PSII antenna must be structurally similar. It is, therefore, apparent that the interpretation of previous studies employing lipase modification of membrane structure was confounded by the lack of specificity of the experimental approach (8, 22).

Effects of High Temperature on Fluorescence. Several authors have interpreted increases in Chl fluorescence which occur upon heating of leaves as an indicator of temperature-induced changes of photosynthetic membrane stability (4, 17, 25). The heat-induced rise in F_o which has been interpreted in terms of a breakdown in energy transfer from LHCP antenna pigments to PSII centers and related inhibition of photochemistry, has been taken as an indicator of the thermal stability of the PSII pigment system. More precisely, the fluorescence rise has been attributed to the physical separation of the LHCP from the PSII core, thereby blocking excitation energy transfer and leading to reemission of excitation energy from LHCP as fluorescence (4). The temperature at which enhanced fluorescence occurs may vary in

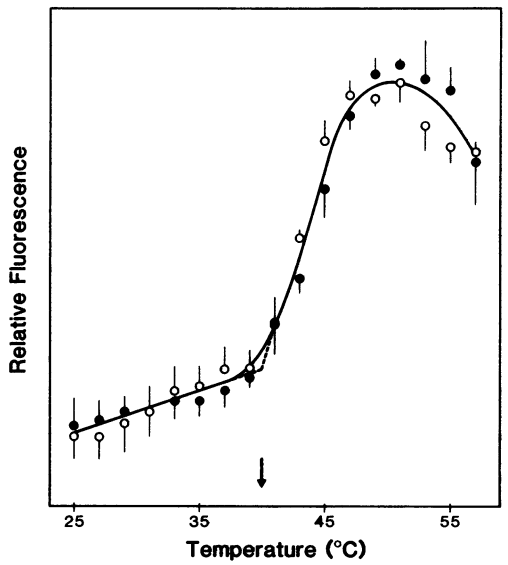

FIG. 6. Temperature-induced fluorescence enhancement yield (F_o) of wild-type (●) and mutant (○) leaves. Plants were grown at 21°C. The arrow indicates the threshold temperature at which fluorescence is enhanced. Each point represents the mean ± SE (*n* = 4).

response to environmental adaptation and appears to be affected by the lipid environment in which the proteins are embedded (17). In this respect, Chl fluorescence may be considered an intrinsic probe of lipid-protein interaction.

The effect of temperature on mutant and wild-type leaves was measured by appressing leaves to a temperature-controlled metal block and measuring fluorescence continuously as the temperature of the heating block was increased from 25 to 56°C at a rate of about 1.5°/min. At approximately 37 to 38°C a transition in the level of fluorescence was observed in both wild-type and mutant leaves (Fig. 6). This response is similar to that observed by comparable experimental approaches with other species (4, 17, 25). There was no significant difference in the threshold temperature or magnitude of the fluorescence response of the mutant as compared to the wild type. Thus, it does not appear that the absence of *trans*-$C_{16:1}$-PG has a significant effect on the thermal stability of the LHCP-PSII core association.

DISCUSSION

The normal growth, chloroplast ultrastructure, and rate of photosynthesis in the *fadA* mutant grown under standard conditions indicates that the role of *trans*-$C_{16:1}$ is subtle (5). The most striking effect attributable to the mutation is the relatively reduced amount of LHCP oligomer which was recovered from mutant membranes following detergent-mediated thylakoid solubilization (Figs. 1 and 2). This observation suggests a role for *trans*-$C_{16:1}$-PG in stabilizing the LHCP oligomer. The simplest hypothesis to explain the apparent instability of LHCP oligomer in the mutant would seem to be that PG containing *trans*-$C_{16:1}$ is more effective at preventing SDS from penetrating the subunit contact sites of the LHCP oligomer than PG containing palmitic acid (*i.e.* 16:0). This is consistent with the results from previous studies showing that phospholipase-A2 treatment of membranes leads to loss of the LHCP oligomer (23), and with studies showing that the LHCP oligomer extracted from SDS gels appears to be specifically enriched in PG containing *trans*-$C_{16:1}$ (28). The results of experiments in which the presence of *trans*-$C_{16:1}$-PG in artificial liposomes enhanced the rate (but not the amount) of reconstitution of LHCP oligomer (24) also supports the concept that the lipid facilitates formation or stability of the LHCP oligomer. However, these observations are difficult to interpret since, for example, the addition of Triton- X-100 to the SDS-solubilization buffer was also reported to increase the amount of $LHCP^1$ at the expense of $LHCP^3$ (1). Similarly, the solubilization of thylakoids with the nonionic detergent octyl-β,D-glucoside rather than with SDS resulted in loss of the apparent *trans*-$C_{16:1}$-PG/$LHCP^1$ association (12). Also, analysis of the lipid composition of mechanically isolated stroma and grana lamellae revealed that the stroma lamellae, which contained low amounts of $LHCP^1$ had higher levels of *trans*-$C_{16:1}$-PG than PSII granal vesicles (10). Thus, we suggest that the effect of the loss of *trans*-$C_{16:1}$-PG on $LHCP^1$ stability may reflect a nonspecific change in the overall properties of the photosynthetic lamellae rather than a specific effect on LHCP *per se*. The observation that the CP1a oligomer is also less stable in the mutant (Fig. 1) lends credence to this view.

It is well established that cations stimulate thylakoid stacking (13), a process which involves LHCP (27). This stimulated consideration of the possibility that the differential cation enhancement of SDS-mediated dissociation of LHCP oligomer in the mutant was related to properties of the LHCP oligomer involved in bringing about membrane appression (2). We analyzed the effect of cations on LHCP function by measuring the effect of cation concentration on the efficiency of exciton transfer from LHCP to PSI (Fig. 5). In principle, cation-induced dissociation of LHCP oligomers might be expected to lead to less efficient exciton transfer to PSI and, therefore, to increased fluorescence from LHCP at 685 nm. Although both mutant and wild type showed a change in the ratio of PSI to PSII fluorescence, there was no significant difference between the two lines at any cation concentration. The absence of a differential effect of NaCl on the ratio of Chl fluorescence at 685 and 734 nm is considered evidence against an important role for *trans*-$C_{16:1}$ in conferring unique functional properties to the LHCP oligomer *in vivo*. Similarly, the absence of a differential effect of divalent cations on fluorescence induction kinetics in mutant *versus* wild type (Table I) indicated that the photochemical efficiency of PSII reaction centers are indistinguishable in the two lines. The effect of NaCl on the proportion of Chl found in $LHCP^1$ is, therefore, probably due to a stimulation of the activity of SDS rather than a specific effect on LHCP quaternary structure. We must conclude that the lipid has no significant *in vivo* effect on LHCP quaternary structure.

The apparent absence of an effect of the *fadA* mutation on PSI or PSII activity contrasts with the results of experiments involving lipase treatment of thylakoid membranes which were designed to examine the role of *trans*-$C_{16:1}$. The lipase treatment was intended to exploit the head group and positional specificity of phospholipase A2 to catalyze preferential removal of the acyl group at position two of PG and phosphatidyl choline (22). In one study, phospholipase-A2 treatment increased the amount of light required to saturate the Hill reaction, decreased the variable fluorescence and increased the time required to reach maximal fluorescence (8). However, the implications of these observations were disputed by Rawyler and Siegenthaler (22) who showed that both PG and phosphatidyl choline were affected by phospholipase A2 to varying degrees, depending on the source of enzyme, and that PSII activity was severely depressed by phospholipase treatment. Whatever the reason for the effects of the lipase treatments the discrepancy between the functional properties of lipase-treated thylakoids and those of the *fadA* mutant illustrate the limited utility of lipolytic analysis in attempting to determine the functional significance of specific acyl groups.

The results of several studies have provided evidence that membrane lipid composition may exert an important influence on the stability of the association of LHCP with the PSII core (4, 17, 25). Indeed, on the basis of correlations between adaptive changes in lipid composition and the threshold for temperature-induced fluorescence, it has been suggested that *trans*-$C_{16:1}$-PG could play a role in mediating thermal stability of the LHCP-PSII complex (17). However, the absence of any differential effect of the *fadA* mutation on the threshold temperature for temperature-induced fluorescence (Fig. 6) renders a specific role for *trans*-$C_{16:1}$ in thermal adaptation untenable.

In conclusion, although we have independently reproduced the evidence for an effect of *trans*-$C_{16:1}$ on *in vitro* LHCP oligomer stability, we have not observed any functional significance associated with the absence of *trans*-$C_{16:1}$. On this basis we propose that *trans*-$C_{16:1}$-PG normally has no effect on the function of the photosynthetic lamellae. We propose that the role is either restricted to an unusual environmental circumstance that we have not investigated, or to a specific phase of development. For example, since *trans*-$C_{16:1}$ accumulates concomitantly with LHCP accumulation it seems possible that it facilitates insertion of proteins into the thylakoid membranes and thereby leads to a more efficient membrane assembly process. The observation that the rate of $LHCP^1$ formation is enhanced in liposomes containing *trans*-$C_{16:1}$ (24) may be considered preliminary evidence in favor of this concept. Whatever the precise role, it seems apparent that the lipid is an element of the fine tuning mechanisms (6) which have evolved to optimize the efficiency of photosynthetic electron transport.

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