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Unsaturation of the membrane lipids of chloroplasts stabilizes the photosynthetic machinery against low-temperature photoinhibition in transgenic tobacco plants

(fatty acid/glycerol-3-phosphate acyltransferase/phosphatidylglycerol/transformation)

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ABSTRACT **Using tobacco plants that had been transformed with the cDNA for glycerol-3-phosphate acyltransferase, we have demonstrated that chilling tolerance is affected by the levels of unsaturated membrane lipids. In the present study, we examined the effects of the transformation of tobacco plants with cDNA for glycerol-3-phosphate acyltransferase from squash on the unsaturation of fatty acids in thylakoid membrane lipids and the response of photosynthesis to various temperatures. Of the four major lipid classes isolated from the thylakoid membranes, phosphatidylglycerol showed the most conspicuous decrease in the level of unsaturation in the transformed plants. The isolated thylakoid membranes from wild-type and transgenic plants did not significantly differ from each other in terms of the sensitivity of photosystem II to high and low temperatures and also to photoinhibition. However, leaves of the transformed plants were more sensitive to photoinhibition than those of wild-type plants. Moreover, the recovery of photosynthesis from photoinhibition in leaves of wild-type plants was faster than that in leaves of the transgenic tobacco plants. These results suggest that unsaturation of fatty acids of phosphatidylglycerol in thylakoid membranes stabilizes the photosynthetic machinery against low-temperature photoinhibition by accelerating the recovery of the photosystem II protein complex.**

The sensitivity of higher plants to chilling is closely correlated with the degree of unsaturation of the fatty acids in the thylakoid membranes of their chloroplasts (1–4). We have demonstrated that both the unsaturation of thylakoid membrane lipids and chilling sensitivity are significantly affected upon transformation of tobacco plants with cDNAs for glycerol-3-phosphate acyltransferases from squash and *Arabidopsis* (5). In particular, the extent of unsaturation of phosphatidylglycerol (PG) was most effectively modified, and this change appears responsible for modification of the ability to tolerate low temperatures.

Photosynthesis at low temperature is impeded when plants are exposed to light (6). This phenomenon is known as low-temperature photoinhibition. The main target for photoinhibition is the photosystem (PS) II protein complex (7). Impairment of electron transport is caused by irreversible damage to the D1 protein, which is one of the heterodimeric polypeptides of the PS II reaction center complex (8, 9).

Using a cyanobacterial transformation system (10–13), we have demonstrated that a decrease in the unsaturation of membrane lipids by mutation of fatty-acid desaturases enhances the sensitivity to chilling of cyanobacterial cells. This phenomenon is explained by the depression of photoinhibition *in vivo* as a result of the unsaturation of membrane lipids. This inference was confirmed in another cyanobacterial system in which the unsaturation of membrane lipids was increased by introducing a gene for desaturases (14). The present study was designed to determine whether the same mechanism as demonstrated in cyanobacteria is operative in the chloroplasts of higher plants.

To study the relationship between the unsaturation of thylakoid membrane lipids and low-temperature photoinhibition in higher plants, we employed transgenic tobacco plants in which the extent of unsaturation of thylakoid membrane lipids was decreased as a result of overexpression of the glycerol-3-phosphate acyltransferase gene from squash, under the control of the cauliflower mosaic virus 35S promoter. We found that the extent of unsaturation of PGs in thylakoid membranes did not affect the process of inactivation that is associated with photoinhibition but regulates the process of recovery of photosynthesis from photoinhibition.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Wild-type and transgenic tobacco plants (*Nicotiana tabacum* var. Samsun) were obtained as described (5). One transgenic tobacco plant, designated Rbcs-SQ, had been transformed with the cDNA for glycerol-3-phosphate acyltransferase from squash, which had been ligated into plasmid pBI-121. The wild type and another transgenic plant, termed pBI-121, into which only the pBI-121 vector had been introduced, were used as controls. These transgenic tobacco plants were self-pollinated and the seeds obtained were allowed to germinate on agar in the presence of 50 μg of kanamycin per liter. Seeds of wild-type plants were germinated in the absence of kanamycin. After growth for 2.5 months, plants were transferred to vermiculite supplemented with a fertilizer (Hyponex; Hyponex, Marysville, OH) and then were transplanted to soil. The plants were grown at day/night temperatures of 28/25°C in natural daylight with additional illumination from incandescent lamps from 04:00 to 07:00 and from 16:00 to 22:00. After about 3 months of growth, mature leaves from the wild-type, pBI-121, and Rbcs-SQ plants were harvested for experiments.

Isolation of Thylakoid Membranes. Twenty grams of leaves from tobacco plants was homogenized in a blender for 30 sec with 200 ml of 50 mM sodium/potassium phosphate buffer (pH 7.4) that contained 20 mM NaCl, 100 mM sucrose, and 1.0 M betaine (*N*-tetramethylglycine). The homogenate was fil-

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Abbreviations: Chl, chlorophyll; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PBQ, phenyl-*p*-benzoquinone; PG, phosphatidylglycerol; PS, photosystem; SQDG, sulfoquinovosyl diacylglycerol.

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tered through three layers of Miracloth (Calbiochem) and centrifuged at 120 × *g* for 10 min. The supernatant was centrifuged at 6000 × *g* for 10 min, and the pellet was suspended in 30 ml of 50 mM sodium/potassium phosphate buffer (pH 7.4) that contained 200 mM NaCl, 300 mM sucrose, and 1.0 M betaine and was subjected to centrifugation at 1000 × *g* for 5 min. The supernatant was recentrifuged again at 6000 × *g* for 10 min, and the pelleted thylakoid membranes were resuspended in 1.0 ml of 25 mM Mes buffer, adjusted with NaOH to pH 6.5, that contained 10 mM NaCl, 300 mM sucrose, and 1.0 M betaine (buffer A) to give a final concentration of chlorophyll (Chl) of 1 mg·ml^{-1}. Membranes were stored in liquid nitrogen prior to analysis.

Analysis of Lipids in Thylakoid Membranes. Lipids were extracted from the isolated thylakoid membranes by the method of Bligh and Dyer (15) with trivial modifications. Classes of polar lipids were fractionated by ion-exchange column chromatography, by column chromatography on silica gel, and by thin-layer chromatography on silica gel, essentially as described (1). The fractionated lipid samples were subjected to methanolysis, and the resultant methyl esters were analyzed by GLC as described (1).

Exposure of Thylakoid Membranes to Light. Thylakoid membranes corresponding to 10 μg of Chl were suspended in 1 ml of buffer A. The suspension was illuminated at 5°C, 15°C, or 25°C in a temperature-controlled reaction vessel with actinic light of various intensities provided by an incandescent lamp in combination with a yellow optical filter (Y-46; Hoya Glass, Tokyo) and an infrared absorbing filter (HA-50; Hoya Glass). The intensity of light was regulated in the range of 0–2.0 mmol·m^{-2}·sec^{-1} by inclusion of various neutral-density filters (Hoya Glass).

Measurement of Photosynthetic Activities. Photosynthetic evolution of oxygen from thylakoid membranes was monitored with a Clark-type oxygen electrode. The oxygen-evolving activity of thylakoid membranes due to the activity of PS II was measured at 25°C (unless otherwise stated) with 0.3 mM phenyl-*p*-benzoquinone (PBQ) as the electron acceptor. Actinic light at an intensity of 1.7 mmol·m^{-2}·sec^{-1} was provided by an incandescent lamp in combination with a yellow optical filter (Y-46) and an infrared absorbing filter (HA-50). The Chl concentration of thylakoid membranes was determined by the method of Arnon (16) and adjusted to about 10 μg of Chl per ml.

Table 1. Composition of lipid classes in thylakoid membranes isolated from wild-type and transgenic tobacco plants

Origin of thylakoid membranes	Lipid class, mol %			
	MGDG	DGDG	SQDG	PG
Wild type	55	32	5	8
pBI-121	53	34	6	7
Rbcs-SQ	57	31	5	7

The values are the means of results from three independent experiments. The deviation of values was <3% in every case.

Exposure of Leaf Disks to Light. Disks of 10 cm^2, cut from leaves of about 6 cm × 10 cm, were placed individually in a temperature-controlled chamber equipped with an oxygen electrode (LD2; Hansatech, Norfolk, U.K.). To induce photoinhibition, the leaf disk was illuminated by light from an incandescent lamp with an infrared absorbing filter (HA-50). The photosynthetic evolution of oxygen by the leaf disk was measured upon illumination at an intensity of 1.5 mmol·m^{-2}·sec^{-1}. A mixture of 5% (vol/vol) CO_2 in water-saturated air was supplied to each leaf disk before measurements were made.

RESULTS

Changes in Lipids of Thylakoid Membranes. Lipids were extracted from isolated thylakoid membranes and separated into lipid classes—namely, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG); and PG. Table 1 shows that there were insignificant changes in the relative levels of lipid classes among the wild-type, pBI-121, and Rbcs-SQ plants. PG accounted for a small proportion—namely, <10%—of the total polar lipids in the three types of plant. Consistent with our previous results (5), these data indicate that overexpression of the acyltransferase from squash had no significant effect on the relative levels of lipid classes.

The results of fatty acid analysis revealed that transformation with only the vector plasmid pBI-121 did not affect the fatty acid composition of the glycerolipids in thylakoid membranes (Table 2). However, the fatty acid composition of the lipids from the thylakoid membranes of Rbcs-SQ transgenic plants was different from those from the wild-type and pBI-121

Table 2. Fatty acids in polar lipids from thylakoid membranes isolated from wild-type and transgenic tobacco plants

	Fatty acid, mol %									
						18:1				
Lipid class in membranes	16:0	16:1*c*	16:1*t*	16:3	18:0	9-*cis*	11-*cis*	18:2	18:3	Σ cis-unsat. FA, mol %
Wild type										
MGDG	3	t	0	5	t	t	t	4	88	97
DGDG	12	t	0	1	3	1	t	2	80	85
SQDG	45	t	0	t	4	2	t	4	45	51
PG	32	0	32	0	3	7	1	10	15	33
pBI-121										
MGDG	3	t	0	7	1	1	t	3	85	96
DGDG	14	t	0	1	3	1	t	3	78	83
SQDG	43	t	0	t	5	2	t	5	45	52
PG	28	0	32	0	4	8	1	11	16	36
Rbcs-SQ										
MGDG	4	t	0	7	1	t	t	1	86	95
DGDG	18	t	0	1	5	t	t	1	74	77
SQDG	55	t	0	0	5	1	t	1	38	40
PG	46	0	34	0	8	1	1	3	7	12

16:0, Hexadecanoic acid (palmitic acid); 16:1*c*, 9-*cis*-hexadecenoic acid (palmitoleic acid); 16:1*t*, 3-*trans*-hexadecenoic acid; 18:0, octadecanoic acid (stearic acid); 18:1, 9-octadecenoic acid (oleic acid) and 11-octadecenoic acid (vaccenic acid); 18:2, 9,12-octadecadienoic acid (linoleic acid); 18:3, 9,12,15-octadecatrienoic acid (α-linolenic acid); t, trace (less than 0.5%). Σ cis-unsat. FA is the sum of the *cis*-unsaturated fatty acids. The values are the means of results obtained from three independent experiments.

Table 3. Oxygen-evolving activity at 10°C, 20°C, and 30°C of thylakoid membranes isolated from wild-type and transgenic tobacco plants

Strain	O_2 evolution, μmol·(mg of Chl)$^{-1}$·hr^{-1}		
	10°C	20°C	30°C
Wild type	97 ± 4	177 ± 6	289 ± 5
pBI-121	98 ± 5	185 ± 5	301 ± 6
Rbcs-SQ	91 ± 5	176 ± 4	295 ± 5

Activities were measured in terms of the PS II-mediated transport of electrons with PBQ as the electron acceptor. The values show the means and range of results from two independent experiments.

plants: the relative levels of saturated 16-carbon fatty acid (designated 16:0) in DGDG, SQDG, and PG increased. In particular, PG was most significantly affected. The relative levels of total cis-unsaturated fatty acids in PG declined from 33% in the wild-type to 12% in the Rbcs-SQ plants.

Activity of PS II in Isolated Thylakoid Membranes. To examine whether changes in unsaturation of fatty acids in PG might alter the rate of photosynthetic electron transport, we compared the rate of evolution of oxygen from thylakoid membranes from the three strains of tobacco plants at different temperatures using PBQ as the electron acceptor (Table 3). The electron transport activities were almost identical despite the differences in levels of unsaturated fatty acids in thylakoid membranes from the three types of plant.

Temperature-Dependent Inactivation of PS II in Isolated Thylakoid Membranes. Fig. 1 compares the profiles of inactivation at various temperatures of the oxygen-evolving machinery when isolated thylakoid membranes were incubated in darkness at high and low temperatures. When the thylakoid membranes were incubated for 20 min, inactivation was observed on the high-temperature side. When membranes were incubated for 12 hr, inactivation was observed on both the low-temperature and the high-temperature sides.

Photoinhibition in Isolated Thylakoid Membranes. Fig. 2 shows the dependence on time of the photoinhibition at 5°C, 15°C, and 25°C of oxygen evolution by thylakoid membranes from wild-type and Rbcs-SQ transgenic plants. The extent of photoinhibition was identical in the thylakoid membranes from the two sources. Fig. 3 shows the dependence on light intensity of photoinhibition at 5°C, 15°C, and 25°C of oxygen evolution by thylakoid membranes from wild-type and Rbcs-SQ transgenic plants. With increases in the intensity of light, the extent of photoinhibition increased. However, there was no difference in the extent of photoinhibition between the thylakoid membranes from wild-type and Rbcs-SQ transgenic plants.

The results in Figs. 2 and 3 lead us to conclude that the extent of photoinhibition in isolated thylakoid membranes is hardly affected by the temperature at which the photoinhibitory treatments were performed. The results also indicate that the unsaturation of fatty acids in PG does not affect the photoinhibition of PS II activity in isolated thylakoid membranes. These are consistent with those reported with *Arabidopsis* (17) and for isolated cyanobacterial membranes in which modifi-

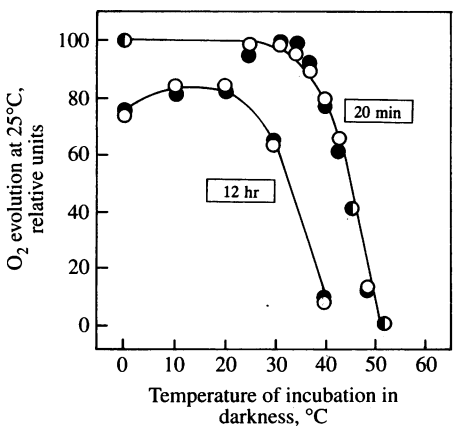

FIG. 1. Profiles of the inactivation of PS II-mediated transport of electrons during incubation in darkness of thylakoid membranes from wild-type (○) and Rbcs-SQ transgenic (●) tobacco plants at various temperatures. The thylakoid membranes (10 μg of Chl per ml) were incubated at the designated temperature in darkness for 12 hr or 20 min in 1.0 ml of buffer A. The activity of PS II-mediated transport of electrons from H_2O to PBQ was measured at 25°C as described in text. The activities before incubation were taken as 100 arbitrary units, which corresponded to 280 ± 15 and 270 ± 10 (μmol of O_2)·(mg of Chl)$^{-1}$·hr^{-1} for thylakoid membranes from wild-type and Rbcs-SQ plants, respectively. The values were calculated from results of three independent experiments. The deviation of values was <7% in each case.

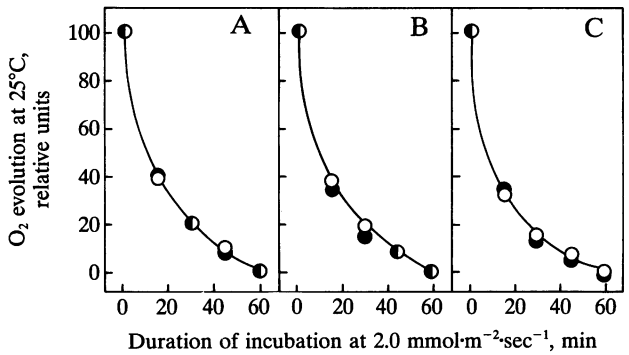

FIG. 2. Photoinhibition of the PS II-mediated transport of electrons in thylakoid membranes isolated from wild-type (○) and Rbcs-SQ transgenic (●) plants. Thylakoid membranes (10 μg of Chl per ml) were incubated in 1.0 ml of buffer A at 5°C (*A*), 15°C (*B*), or 25°C (*C*) with irradiation at 2.0 mmol·m^{-2}·sec^{-1}. Then the transport of electrons from H_2O to PBQ was monitored at 25°C as described in text. The absolute values for evolution of oxygen before incubation were taken as 100 arbitrary units, which corresponded to 280 ± 15 and 270 ± 10 (μmol of O_2)·(mg of Chl)$^{-1}$·hr^{-1} in thylakoid membranes from the wild-type and Rbcs-SQ plants, respectively. The values were calculated from results of three independent experiments. The deviation of values was <7% in each case.

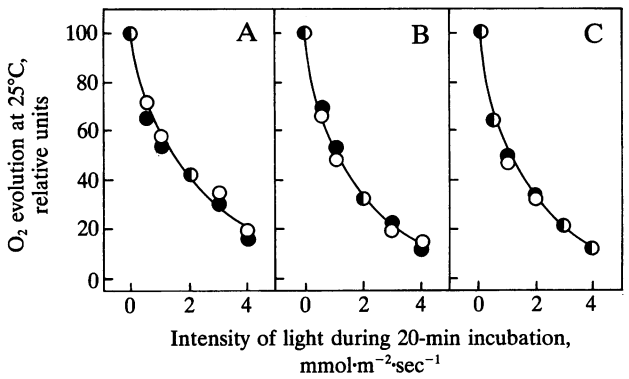

FIG. 3. Effects of the intensity of light on the photoinhibition of the PS II-mediated transport of electrons in thylakoid membranes from wild-type (○) and Rbcs-SQ transgenic (●) plants. Thylakoid membranes (10 μg of Chl per ml) were incubated in 1.0 ml of buffer A at 5°C (*A*), 15°C (*B*), or 25°C (*C*) for 20 min under light at various intensities. Then the transport of electrons from H_2O to PBQ was monitored at 25°C, as described in text. The absolute values for the evolution of oxygen before incubation were taken as 100 arbitrary units, which corresponded to 280 ± 15 and 270 ± 10 (μmol of O_2)·(mg of Chl)$^{-1}$·hr^{-1} for thylakoid membranes from the wild-type and Rbcs-SQ tobacco plants, respectively. The values are means from two independent experiments. The range of values was <7%.

cation by genetic engineering of levels of unsaturation of membrane lipids failed significantly to affect susceptibility to photoinhibition (10, 11).

Photoinhibition of Photosynthesis in Leaves. The extent of photoinhibition *in vivo* results from the outcome of competition between the photoinduced process of inactivation and the counteracting recovery process (18). Therefore, we attempted to compare the extent of photoinhibition in a system *in vivo*—namely, in leaves—in which both the inactivation process and the recovery process were operative. Leaf disks were illuminated at an intensity of 0.6 mmol·m^{-2}·sec^{-1} for designated periods at 5°C, 15°C, and 25°C. Then, after incubation for 20 min in darkness to bring the temperature of the sample to 25°C, the rate of photosynthetic evolution of oxygen was measured. Fig. 4 *A–C* shows that, in the absence of lincomycin, photoinhibition was more significant at low temperature than at high temperature in both wild-type and Rbcs-SQ plants. However, this tendency was more marked in the Rbcs-SQ than wild-type plants. At 5°C, the extent of photoinhibition during 10 hr of illumination showed a remarkable difference between wild-type and Rbcs-SQ plants. These results indicate that unsaturation of fatty acids of PG in thylakoid membranes stabilizes the photosynthetic machinery against photoinhibition.

It is possible to isolate the photo-induced inactivation process from the recovery process if the protein synthesis is blocked by an inhibitor, such as lincomycin, of translation in prokaryotes. Fig. 4 *D–F* shows that the presence of lincomycin accelerated photoinhibition and eliminated the effect of temperature and the difference between wild-type and Rbcs-SQ plants. These observations confirm that the photo-induced inactivation process of photoinhibition is not affected by the unsaturation of PG.

Recovery of Photosynthesis After Low-Temperature Photoinhibition. Fig. 5 shows the recovery of photosynthesis after low-temperature photoinhibition in leaves of wild-type, pBI-121, and Rbcs-SQ plants. Leaf disks were illuminated at low temperature with high-intensity light to decrease the rate of

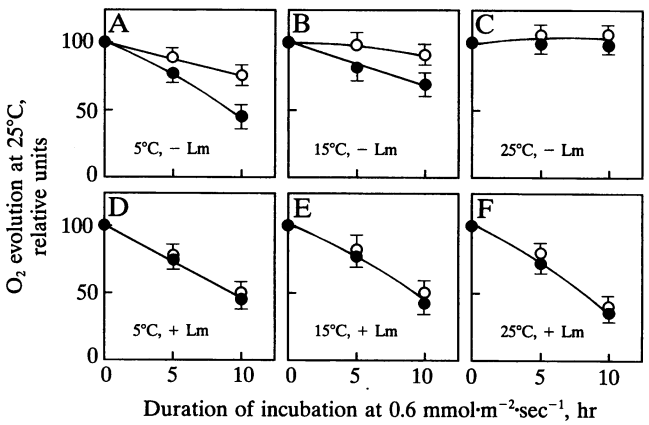

FIG. 4. Extent of photoinhibition of photosynthesis in leaves of wild-type (○) and Rbcs-SQ transgenic (●) tobacco plants in the presence (+ Lm) and absence (− Lm) of lincomycin. Leaf disks were illuminated at a light intensity of 0.6 mmol·m^{-2}·sec^{-1} at the designated temperatures. Lincomycin was administered to a leaf blade through a cut end of the petiole during transpiration by immersing the petiole in 0.6 mM lincomycin for 4 hr at 25°C at a light intensity of 0.15 mmol·m^{-2}·sec^{-1}. The oxygen-evolving activity was measured at 25°C 10 min after incubation in darkness. The activity is expressed as the percentage of activity before photoinhibition. The rate of oxygen evolution at 25°C corresponding to 100 arbitrary units was 0.036 ± 0.003 and 0.034 ± 0.003 (ml of O_2)·min^{-1}·(10 cm^2)$^{-1}$ in the wild-type plants and in the Rbcs-SQ tobacco plants, respectively, in the presence or absence of lincomycin. The values were obtained from the results of three independent experiments.

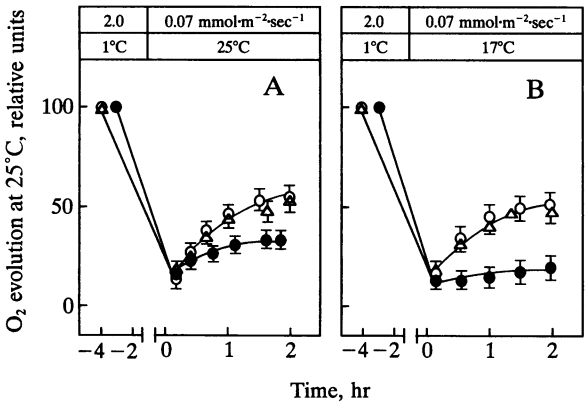

FIG. 5. Recovery of the photosynthetic machinery from photoinhibition in leaves of wild-type (○), pBI-121 (△), and Rbcs-SQ (●) plants. Leaf disks from the transgenic plants were illuminated at 1°C at an intensity of 2.0 mmol·m^{-2}·sec^{-1} for 3 or 4 hr to reduce the rate of photosynthesis to about 20% of the original rate. Then the disks were incubated at 25°C (*A*) or 17°C (*B*) in light at an intensity of 0.07 mmol·m^{-2}·sec^{-1}. The initial photosynthetic evolution of oxygen, measured at 25°C (*A*) and 17°C (*B*), was taken as 100 arbitrary units and corresponded to 0.036 ± 0.003 and 0.021 ± 0.004 (ml of O_2)·min^{-1}·(10 cm^2)$^{-1}$, respectively, for the wild-type plants; 0.037 ± 0.003 and 0.021 ± 0.004 (ml of O_2)·min^{-1}·(10 cm^2)$^{-1}$, respectively, for the pBI-121 plants; and 0.034 ± 0.003 and 0.022 ± 0.003 (ml of O_2)·min^{-1}·(10 cm^2)$^{-1}$, respectively, for the Rbcs-SQ plants. The values were calculated from results of three independent experiments.

evolution of oxygen to about 20% of the original rate. Then recovery was monitored at 25°C and 17°C under dim light. Under these conditions, the rate of photosynthesis in wild-type and pBI-121 plants returned to 50–60% of the original rate after 2 hr. However, the recovery of the photosynthetic machinery in the Rbcs-SQ plants at 25°C was slower than that in the wild-type plants. At 17°C, recovery was almost negligible in the Rbcs-SQ plants.

DISCUSSION

Elimination of cis-Unsaturated PG Molecules by Gene Manipulation. This study was conducted to examine the effect of unsaturation of the fatty acids in thylakoid membrane lipids on the chilling tolerance of the photosynthetic machinery, taking advantage of transgenic tobacco plants that had been transformed with cDNA for glycerol-3-phosphate acyltransferase. For this purpose, we analyzed the lipids and fatty acids in isolated thylakoid membranes. This analysis provided more informative evidence than did the analysis of lipids that had been directly extracted from leaves (5) when the unsaturation of membrane lipids was correlated with the responses of photosynthesis to various temperatures. We found that the transgenic tobacco plants had a significantly depressed level of cis-unsaturated PG in their thylakoid membranes.

Effect of Unsaturation of PG on the Activity of PS II. To determine whether the unsaturation of PG might affect the temperature-related stability of the photosynthetic machinery, we studied the PS II-mediated transport of electrons in thylakoid membranes at different temperatures (Table 3). We observed no significant differences in PS II activity at 10°C, 20°C, and 30°C between the thylakoid membranes from wild-type and Rbcs-SQ transgenic plants. In our earlier studies (11, 19), we failed to find any significant differences in temperature-related responses of the photosynthetic machinery in cyanobacterial membranes, in which the unsaturation of membrane lipids had been manipulated by disruption of genes for desaturases (13, 20).

Effect of Unsaturation of PG on the Stability of PS II. We previously demonstrated that the unsaturation of membrane

lipids in cyanobacteria had no effect on the thermal stability of the photosynthetic machinery (14, 19). In agreement with these earlier results, the experimental observations in the present study (Fig. 1) demonstrated that in higher plants the unsaturation of PGs of thylakoid membranes had no effect on the thermal stability of the oxygen-evolving activity. However, they stand in marked contrast to an earlier hypothesis, based on physiological studies (21), that saturation of membrane lipids stabilizes the machinery for the photosynthetic evolution of oxygen against inactivation by heat. Pearcy (22) and Raison *et al.* (21) observed that the thermal stability of the photosynthetic machinery increased in parallel with the saturation of the fatty acids in the lipids of the thylakoid membrane. However, modification of the extent of unsaturation and thermal stability of photosynthetic machinery in their experiments were achieved by changes in growth temperature, which could affect levels of many additional metabolites and enzymes (23–26). Therefore, there seems to be no reason to correlate a change in thermal stability directly with the extent of saturation of membrane lipids.

Effect of Unsaturation of PG on the Inactivation Associated with Photoinhibition. Photoinhibition includes photo-induced damage to the D1 protein in the PS II complex (9, 27, 28). The damaged D1 protein is degraded and removed from the PS II complex, and newly synthesized D1 protein is inserted into the PS II complex to restore the photochemical activity (27). Therefore, it can be argued that the extent of photoinhibition *in vivo* corresponds to the outcome of the competition between the photo-induced damage to the D1 protein in the PS II complex and the reassembly of the PS II complex with newly synthesized D1 protein (18). From this perspective, the protective effect of unsaturation of PG on photoinhibition can be analyzed in terms either of suppression of the photo-induced inactivation of the PS II complex or of acceleration of the recovery of the PS II complex.

To determine whether the unsaturation of fatty acids in PG prevents the inactivation process, we separated the inactivation process from the recovery process that is associated with photoinhibition. This separation was achieved by using isolated thylakoid membranes and leaf disks treated with lincomycin in which the recovery process was no longer operative (Figs. 2 and 3). No significant difference in the rate of inactivation was observed between thylakoid membranes from wild-type and transgenic plants. These results imply that the unsaturation of fatty acids in thylakoid membrane lipids does not affect the process of inactivation that leads to the photoinhibition of photosynthesis and the activity of PS II.

Acceleration of the Recovery from Photoinhibition by Increased Unsaturation of PG. We demonstrate in the present study that Rbcs-SQ transgenic plants were more susceptible to photoinhibition than wild-type and pBI-121 plants. This result can be explained if the unsaturation of fatty acids in PG accelerates the recovery process. This possibility was confirmed by the observation that the extent of the recovery of the photosynthetic machinery from the low-temperature photoinhibition in Rbcs-SQ transgenic plants was lower than that in the wild-type and pBI-121 plants (Fig. 5). These results strongly suggest that the unsaturation of PG can play a determinative role in protection of the photosynthetic machinery against low-temperature photoinhibition via acceleration of the recovery process.

Gombos *et al.* (11) demonstrated in a transgenic cyanobacterial system that the unsaturation of membrane lipids does not affect the inactivation process but accelerates the recovery from photoinhibition. Our present results suggest that the unsaturation of membrane lipids affects low-temperature photoinhibition in the same way in both cyanobacteria and higher plants.

The recovery process after photoinduced damage to the PS II reaction center may involve several complicated steps, which could include proteolytic degradation of the damaged D1 protein, removal of the degraded D1 protein, synthesis of the precursor to the D1 protein, processing of the precursor protein, and the reassembly of the PS II complex with the new D1 protein (27). PGs in thylakoid membranes are preferentially involved in protein–lipid interactions (29, 30). Thus, it is quite probable that changes in the extent of unsaturation of fatty acids in PG can modify the molecular environments of the PS II reaction center complex, thereby affecting the turnover of D1 protein in the PS II complex. However, the specific step that is rate-determining and is accelerated by enhanced unsaturation of fatty acids in PG remains to be identified.

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