Small Light-Harvesting Antenna Does Not Protect from Photoinhibition

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ABSTRACT

High-light-induced decrease in photosystem II (PSII) electron transfer activity was studied in high- and low-light-grown pumpkin (Cucurbita pepo L.) plants in vivo and in vitro. The PSII light-harvesting antenna of the low-light leaves was estimated to be twice as big as that of the high-light leaves. The low-light leaves were more susceptible to photoinhibition in vivo. However, thylakoids isolated from these two plant materials were equally sensitive to photoinhibition when illuminated in the absence of external electron acceptors. Only the intensity of the photosynthetic light and the chlorophyll concentration of the sample, not the size of the light-harvesting antenna, determined the rate of PSII photoinhibition in vitro. Because excitation of the reaction center and not only the antenna chlorophyll is a prerequisite for photoinhibition of PSII activity, independence of photoinhibition on antenna size provides support for the hypothesis (Schatz EH, Brock H, Holzwarth AR [1988] Biophys J 54: 397–405) that the excitations of the antenna chlorophylls are in equilibrium with the excitations of the reaction centers. Better tolerance of the high-light leaves in vivo was due to a more active repair process and more powerful protective mechanisms, including photosynthesis. Apparently, some protective mechanism of the high-light-grown plants is at least partially active at low temperature. The protective mechanisms do not appear to function in vitro.

Photoinhibition of photosynthesis (for reviews, see refs. 19 and 24) occurs when a plant is exposed to higher light intensity than experienced during growth. Photoinhibition is characterized by a decrease in PSII activity, quenching of maximum and variable fluorescence, and decrease in the quantum yield of photosynthesis. These symptoms may, however, be due to two processes: protective increase in thermal dissipation of excitation energy among the antenna pigments (for reviews of the mechanisms, see refs. 10 and 19), and photoinhibition of photosynthetic energy conversion in the reaction center.

We concentrate here on the inhibition of the reaction center activity, using the term “photoinhibition” in a narrow sense to describe high-light-induced decrease in the electron transfer activity of PSII. Thermal dissipation of excitation energy at the antenna level should not affect the light-saturated activity. Relaxation of thermal dissipation at the antenna level should also not require de novo protein synthesis, which is known to be essential for recovery from photoinhibition (14). A recent report (20) supports the distinction between two light-induced processes, only one of which lowers PSII activity and requires protein synthesis for recovery.

The sensitivity of a photosynthetic system to photoinhibition is generally thought to be affected by: (a) the rate of energy dissipation by protective mechanisms, including photosynthesis; (b) the rate of concurrent recovery; and (c) the size of the light-harvesting antenna of PSII (19). Shade-grown plants are more susceptible to high light than are plants grown in high light (9), which could be partially due to the all of the above listed factors, including antenna size. Direct evidence about the effect of the antenna size comes from in vitro photoinhibition experiments with Chl b-less mutants that have a reduced light-harvesting antenna (7) and from grana and stroma thylakoid vesicles enriched in PSII known to possess a big or a small light-harvesting antenna, respectively (21). However, the importance of the antenna size per se appears not to be experimentally well established, inasmuch as differences in other factors affecting the sensitivity have not been completely excluded.

In this study, we tested the significance of the size of the light-harvesting antenna in susceptibility of PSII to photoinhibition. The antenna size could be affected in two ways. Photosynthesis reduces excitation density in a small antenna more effectively than in a large one. Also, a large antenna might be able to transfer more excitations to the reaction center than a small one. This effect, however, is important only if excitation transfer from the antenna to the reaction center is much more probable than excitation transfer from the reaction center back to the antenna.

MATERIALS AND METHODS

Pumpkin (Cucurbita pepo L. cv Jättiläismeloni) plants were grown at 22/18°C, in 12-h light (Osmar powerstar HQI-T lamps)/dark rhythm, either at a PPFD of 1000 to 1300 μmol m⁻² s⁻¹ or at 50 to 80 μmol m⁻² s⁻¹. Full-grown leaves of 3- to 5-week-old plants were used in the experiments.

Photoinhibition and Recovery Conditions

The plants were collected at the end of the dark period. The photoinhibition treatments of attached leaves were carried out in a windowed growth chamber, and the RH was kept high by continuously spraying water into the chamber. Four to six leaves were illuminated in every experiment. In the low-temperature treatments, the plants were cooled down to 1°C before switching the photoinhibitory light on. A 1200-W HMI arc lamp (Sylvania Brite Beam) with a color temper-
ature of 5600 K was used as a light source. Variation in the photon flux rate of the illuminated spot (1300–1700 μmol m⁻² s⁻¹) and differences between the illuminated spots were compensated for by taking samples from different locations. The leaf temperature (either 20°C or 2 ± 1°C) was continuously monitored during the inhibition periods with a thermocouple attached to the back side of an illuminated leaf.

For the recovery assays, photoinhibited plants were transferred to a growth chamber to 20°C and a PPFD of 50 μmol m⁻² s⁻¹. To start the recovery from the same degree of photoinhibition, the low-light-grown plants were pretreated at a PPFD of 1500 μmol m⁻² s⁻¹ at 2 ± 1°C for 1.5 h and the high-light-grown plants for 3 h.

The photoinhibition treatments of isolated thylakoids were carried out at 2°C in a beaker immersed in a temperature-controlled water bath. Very slow stirring was applied. A 250-W projector mounted above the beaker was used as a light source, and the photon flux rate (1000, 2000, or 3000 μmol photons m⁻² s⁻¹) was adjusted with neutral density filters (Balzers). The incubation medium contained 50 m Hepes-KOH (pH 7.2), 0.3 m sorbitol, 10 mM NaCl, and 10 mM MgCl₂. The Chl concentration was 0.2 mg/mL or 0.4 mg/mL, as indicated, and the thylakoid suspension layer was about 5 mm thick. Aliquots of the suspension were drawn for both electron transfer activity and fluorescence measurements during the inhibition period. Electron transfer was measured immediately, and fluorescence induction was measured after a dark adaptation time of 3 to 8 min.

### Thylakoid Isolation and Activity Measurements

Thylakoids were isolated from leaves immediately after the treatments with a rapid procedure as described (27). For the in vitro photoinhibition treatments of the thylakoids, the isolation procedure was essentially similar, except that the thylakoids were washed once in the isolation medium, and the final suspension medium did not contain BSA.

The light-saturated activities of PSII (H₂O to PPBQ³) and the whole thylakoid electron transfer chain (H₂O to methyl viologen) were measured with an oxygen electrode (Hansatech, England) as in Aro et al. (3). The purpose of measuring the whole-chain activity was to reveal the functioning of the recovery cycle of PSII (16). During the cycle, the inactive or non-Q₀-PSII (6, 13, 22) is converted into active Q₀-PSII and non-Q₀-PSII, both of which are produced by the incorporation of new D₁ protein into the PSII core. Because PSI activity is not inhibited during photoinhibition of attached pumpkin leaves (27, 28), changes in the whole chain electron transport activity reflect changes in the activity of the active or Q₀-type PSI; while the PSI activity obtained with PPBQ as electron acceptor reflects both non-Q₀- and Q₀-PSII. PPBQ accepts electrons from both types of PSI (17).

³ Abbreviations: PPBQ, phenyl-p-benzoquinone; F₀ and Fₘₐₓ, initial and maximal fluorescence, respectively; Fₛ, the inflection point of the room-temperature fluorescence curve; Q₀b, second stable electron acceptor of PSII; t(P), time to reach the P-peak of fluorescence induction.

### Determination of the Difference in the Antenna Size

The Chl-protein complexes of the thylakoid membranes were separated with PAGE as in Ovaska et al. (23). Solubilization was done with octyl glucoside according to the method of Camm and Green (5). The nondenaturing gels consisted of a 4% stacking gel and a 6 to 25% acrylamide gradient in the separation gel. The gels were scanned at 675 nm with a gel scanner mounted on a Perkin Elmer spectrophotometer. Identification of the peaks was ascertained with measurements of absorbance spectra. Gels were run from two batches of the plants.

Chl was determined in 80% acetone according to the method of Arnon (2).

### Fluorescence Measurements

Fluorescence induction of leaf discs was measured with a pulse amplitude modulated fluorometer (PAM 101; Heinz Walz, Federal Republic of Germany) at room temperature. After measuring F₀, the Kautsky transients were initiated with low actinic light (PPFD 75 μmol m⁻² s⁻¹). Saturating white light (PPFD 5500 μmol m⁻² s⁻¹) was switched on after reaching the intermediary F₁ level, to obtain Fₘₐₓ. Before the fluorescence measurements, the leaf discs were allowed to dark-adapt for 30 min on moist paper in a Petri dish. The long dark adaptation time was applied to allow for relaxation of the fast relaxing components of light-induced fluorescence quenching that are not related to photoinhibition of reaction center activities.

Fluorescence induction of DCMU-poisoned thylakoids was measured with a laboratory-constructed fluorometer as described (27), except that the Chl concentration was 20 μg/mL and the PPFD of the blue exciting light was 50 μmol m⁻² s⁻¹. Fluorescence was monitored for 0.8 to 3 s, until maximal fluorescence was reached. Before measurements, the thylakoids were allowed to dark adapt for 3 to 8 min. The medium contained 50 mM Hepes-KOH, pH 8.0, 0.33 mM sorbitol, 10 mM NaCl, and 5 mM MgCl₂. Three induction curves were recorded from each sample.

The fluorometers were controlled and the data was collected and analyzed with the FIP fluorescence induction software and ADC-12 A/D card (29).

### RESULTS

**Difference in the Antenna Size**

Growth in low light is known to induce a larger light-harvesting antenna of PSII than growth in high light (for a review, see Ref. 1). The Chl a to b ratio is lower in the low-light grown plants, which indicates a higher percentage of the light-harvesting Chl a/b protein of PSI. The ratio of Chl associated with the light-harvesting Chl-protein complexes of PSII (LHClI, CP29, CP27, and CP24) to that associated with the PSI core components (CP47 and CP43) was 8.3 in the low-light-grown plants and 4.0 in the high-light-grown plants (Table I), suggesting that the light-harvesting antenna of the low-light-grown plants was about twice as big as that of the high-light-grown plants.

Fluorescence measurements were done to qualitatively as-
certain that the larger antenna of the low-light-grown plants also results in more efficient light harvesting in low light. The time needed to reach the P-peak, t(P), of room-temperature fluorescence induction of leaf discs was much longer in the high-light grown plants when the PPFD of the exciting light was below 40 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 1).

**High Light Treatments of Attached Leaves at 20°C**

The reactions of attached leaves to high light treatment at 20°C were studied with room-temperature fluorescence induction of leaf discs after 30 min of dark adaptation. As expected, \( F_{\text{max}} \) was lowered much less and soon reached a steady state in the high-light-grown plants (Fig. 2A). \( F_{\text{o}} \) increased in both plant materials during the first 100 min of photoinhibition. After that point, the increase in \( F_{\text{o}} \) continued only in the high-light-grown plants (Fig. 2B).

In the high-light-grown plants, \( F_{\text{T}}-F_{\text{o}} \), a parameter that reflects the amount of inactive PSII (6), started to grow after an initial slight decline, whereas this parameter declined during the inhibition period in the low-light-grown plants (Fig. 2C).

**Figure 1.** t(P) plotted as a function of the PPFD of the exciting light. Fluorescence induction of leaf discs from high- and low-light-grown plants was measured with the pulse amplitude modulated fluorometer after a dark adaptation time of 2 h.

**Table 1.** Chl a to b Ratio and the Ratio of PSII Light Harvesting Components (LHCII, CP29, CP27, CP24) to PSII Core Components (CP47, CP43) of the Leaves of Pumpkin Plants Grown at the PPFD of 50 to 80 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) or 1000 to 1300 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

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<tr>
<th></th>
<th>High-Light-Grown</th>
<th>Low-Light-Grown</th>
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<tbody>
<tr>
<td>Chl a/b ratio</td>
<td>3.8 ± 0.2</td>
<td>3.0 ± 0.0</td>
</tr>
<tr>
<td>Light harvesting/core ratio</td>
<td>4.0 ± 0.3</td>
<td>8.3 ± 0.8</td>
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**Figure 2.** Time-courses of changes in \( F_{\text{max}} \) (A), \( F_{\text{o}} \) (B), and \( F_{\text{T}}-F_{\text{o}} \) (C) of leaf discs punched from leaves of high- (●) and low-light-grown (○) plants during illumination of intact attached leaves at 20°C. Fluorescence was measured after 30 min of dark adaptation. Results from two independent experiments are shown. All values are presented as percent of the control.
**Photoinhibition of Isolated Thylakoids**

When thylakoids isolated from high- and low-light-grown plants were exposed to strong illumination, the rate of photoinhibition was strictly dependent on photon flux density (Fig. 3) in both materials. However, the rate of photoinhibition was completely independent of which plant material the thylakoids had been isolated from. The inhibition of PSII activity followed the same kinetics as the decrease in F<sub>max</sub>. Our later experiments with different PSII preparations confirmed that, irrespective of the intensity of the photoinhibitory illumination, the rate of inhibition of PSII activity does not depend on the antenna size (E-M Aro, E Tyystjärvi, P Mäenpää, manuscript in preparation).

**Photoinhibition of Attached Leaves at 2°C and Recovery from Photoinhibition**

In addition to fluorescence and total PSII activity (H<sub>2</sub>O to PPBQ), the whole chain electron transfer activity (H<sub>2</sub>O to methyl viologen) was measured from plants exposed to high light at low temperature and during subsequent recovery. By comparing PSII and whole chain electron transfer activities, one can get an insight into the mechanism of recovery from photoinhibition in pumpkin (27).

The high-light-grown plants were less susceptible to photoinhibition at 2°C (Fig. 4), although many proposed protective mechanisms like zeaxanthin formation and migration of phosphorylated light harvesting component LHCII are slowed down or inhibited by low temperature (4, 11).

Recovery from photoinhibition was much faster in the high-light-grown plants. While the whole chain electron transfer activity was completely restored within 3 to 6 h after photoinhibition in the high-light-grown plants, fluorescence parameters needed more time for complete recovery (Fig. 5). Also in the low-light-plants, some recovery of the whole chain activity occurred during the first hour, but after that, the recovery continued only very slowly. In both materials, PSII activity recovered at slower rate than whole chain electron transfer activity, but faster than F<sub>max</sub>.

**DISCUSSION**

**Photoinhibition in Vitro is Independent of the Size of the Light-Harvesting Antenna**

The most important result of this study was that photoinhibition of isolated thylakoids without added electron acceptors is completely independent of the size of the light-harvesting antenna. The fact that the overall kinetics of inhibition was determined by the photon flux density indicates that the lack of an antenna size effect was not due to some nonphotoinhibitory side reaction or light-saturation of photoinhibition (Fig. 3).

Photoinhibition of the reaction center complex requires energy transfer from the surrounding antenna. A larger antenna would result in faster photoinhibition if energy transfer from the antenna to the reaction center were unidirectional, because more excitation energy would be transferred to the reaction center from a larger antenna. However, recent evidence from picosecond studies of fluorescence decay suggests that the excitations of antenna Chl are in equilibrium with excitations of the reaction centers (26). If this “shallow trap” model is valid, the lack of an effect of the antenna size is reasonable.

Photoinhibition is one of the reactions that can follow excitation of the reaction center, and the probability of photoinhibition is a function of the state of the reaction center complex. If the excitations of the antenna Chl are in equilibrium with the excitations of the reaction centers (26), then the relative time that a reaction center is in an excited state is proportional to the excitation density of the antenna system. The excitation density, at a given photon fluence rate and Chl concentration, does not depend on the proportions of antenna and reaction center Chls in the sample, if electron transfer does not effectively lower the excitation density.

*In vitro* results of Cleland et al. (8) and Mäenpää et al. (21) seem to show that the smaller antenna size of PSIIβ renders it less sensitive to photoinhibition than PSIIα. However, these results do not exclude the possibility that the characteristics of the reaction center of PSIIβ and possibly the location of
Figure 4. Time-courses of the inhibition of $F_{\text{max}}$ (A), whole chain (WC) electron transfer activity (B), and PSII activity (C) measured from thylakoids isolated from the leaves of high- (●) and low-light grown (○) pumpkin plants after photoinhibition treatments of attached leaves at 2°C. PPFD 1500 μmol m$^{-2}$ s$^{-1}$. Each point represents the results of two to three independent experiments. All values are expressed as percent of the control. se is shown for experimental points where $n = 3$.

Figure 5. Time-courses of the recovery of $F_{\text{max}}$ (A), whole chain (WC) electron transfer activity (B), and PSII activity (C) measured from thylakoids isolated from pumpkin leaves after photoinhibition and recovery treatments of attached leaves. The photoinhibitory pretreatment at the PPFD of 1500 μmol m$^{-2}$ s$^{-1}$ and 2°C lasted 3 h in the high-light-grown plants (●) and 1.5 h in the low-light-grown plants (○), as indicated. Each point represents the average of two to three independent experiments.
PSI\(\beta\) in the stroma thylakoids might make it less sensitive. Tolerance of the reaction center of PSI\(\beta\) to high light is expected since PSI\(\beta\) is probably involved in the repair cycle of PSI (16).

Thylakoids of Chl b-less barley were found to be less susceptible to photo inhibition than those of the wild type (7). However, in vitro experiments (J Leverenz, G Òquist, G Wingsle, manuscript in preparation) show that the leaves of the mutant are more susceptible to high light. The conflict between in vitro and in vivo results suggests that characteristics other than the antenna size determine the sensitivity of the wild and chlorina \(f_2\) type mutant barley to photo inhibition.

Limited protection from photoinhibition in vivo by phosphorylation of PSI polypeptides (18) could be taken as evidence that the size of the light-harvesting antenna is important in the sensitivity to high light. However, protection of thylakoids against photo inhibition in vivo by phosphorylation has not been confirmed.

**Plants Grown at High Light Recover Faster and Possess More Powerful Protective Mechanisms**

The smaller susceptibility of high-light-grown cyanobacteria to photo inhibition has been hypothesized to result from faster concurrent recovery (25) and from faster zeaxanthin-dependent protective dissipation of excess energy (12). In higher plants, faster dissipation of excitation energy through photosynthesis and other nonradiative channels is often thought to explain the smaller sensitivity of high-light-grown plants (9, 15). In pumpkin leaves, recovery was faster in the high-light-grown plants (Fig. 5). The faster recovery, together with more efficient protective mechanisms, explains why they reached a steady \(F_{\text{max}}\) level during illumination at 20°C (Fig. 2). Faster recovery in high-light leaves has also been reported earlier (9), but Greer and Laing (15) found no differences in the rate of recovery between plants grown under different light intensities.

Our previous studies indicate that the recovery cycle of PSI is inhibited by low temperature in pumpkin leaves (3, 27). Still, the high-light-grown plants were less susceptible to photo inhibition even at 2°C. In light of the thylakoid experiments, the better tolerance must be due to photochemical or purely protective nonradiative dissipation of excitation energy. The fact that photosynthesis lowers the excitation density in a small antenna more effectively than in a large one can be largely neglected, because photosynthesis is not very active at 2°C in the chilling-sensitive pumpkin. The zeaxanthin mechanism (for a review, see ref. 10) could be responsible for protective dissipation of excitation energy. The formation of zeaxanthin is slowed down at low temperatures (11), but even then, a large xanthophyll pool that is probably present in the high-light grown plants (10) could be converted to zeaxanthin more efficiently than a small pool of the low-light-grown plants.

The fact that the recovery of fluorescence lags behind the recovery of electron transfer activities (Fig. 5) suggests that a fraction of fluorescence is quenched independently of the loss of the activity of the reaction centers. This protective quenching does not relax at the same rate as the photo inhibited reaction centers are repaired.

The finding that the whole-chain electron transfer activity is restored somewhat faster than the total PSI activity (Fig. 5, B and C) supports our earlier suggestion (27) that during photoinhibition at low temperature, non-Q\(_{b}\)-PSII is not converted to Q\(_{b}\)-PSII. When the plants are transferred to favorable conditions, this conversion increases the whole-chain activity faster than synthesis of new D1 protein increases the total PSI activity. Behavior of the F\(_{v}\)-F\(_{m}\) fluorescence parameter during photoinhibition at room temperature (Fig. 3C) suggests that production of non-Q\(_{b}\)-PSII is faster in the high- than in the low-light-grown plants.

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**LITERATURE CITED**


