Slow Degradation of the D1 Protein Is Related to the Susceptibility of Low-Light-Grown Pumpkin Plants to Photoinhibition[1]

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ABSTRACT

Photoinhibition of photosystem II (PSII) electron transport and subsequent degradation of the D1 protein were studied in pumpkin (*Cucurbita pepo* L.) leaves developed under high (1000 μmol m^{-2} s^{-1}) and low (80 μmol m^{-2} s^{-1}) photon flux densities. The low-light leaves were more susceptible to high light. This difference was greatly diminished when illumination was performed in the presence of chloramphenicol, indicating that a poor capacity to repair photodamaged PSII centers is decisive in the susceptibility of low-light leaves to photoinhibition. In fact, the first phases of the repair cycle, degradation and removal of photodamaged D1 protein from the reaction center complex, occurred slowly in low-light leaves, whereas in high-light leaves the degradation of the D1 protein more readily followed photoinhibition of PSII electron transport. A modified form of the D1 protein, with slightly slower electrophoretic mobility than the original D1, accumulated in the appressed thylakoid membranes of low-light leaves during illumination and was subsequently degraded only slowly.

The D1 protein, one of two heterodimeric polypeptides of the PSII reaction center complex, has the highest turnover rate of all the thylakoid polypeptides (12, 23). In the unicellular alga *Chlamydomonas reinhardtii*, it has been shown that D1 turnover further increases when the cells are exposed to photoinhibitory light (18, 27, 32). In higher plants, the photoinhibition-enhanced turnover of the D1 protein has been questioned (8) because photoinhibition of PSII in intact leaves induces hardly any net decrease in the amount of the D1 protein (8, 15). Photoinhibition-enhanced degradation of the D1 protein in intact leaves can be demonstrated if the leaves are illuminated in the presence of an inhibitor of chloroplast protein synthesis (15). Also [^{14}C]leucine labeling of spruce needles has indicated a high turnover rate of the D1 protein in the light (11).

Degradation of the D1 protein in vivo is preceded by a modification that leads to a slower electrophoretic mobility of the protein (5, 15). The modified form, D1^{*2}, has been shown to be a phosphorylated form of the D1 protein (3, 10).

Furthermore, we have shown that D1* is actually degraded more slowly than the original D1 when isolated thylakoids are illuminated in the presence of ATP (3), suggesting that the modification to D1* may be involved in controlling the degradation.

Plants differ in their susceptibility to photoinhibition, and this is, at least partially, dependent on their capacity to repair photodamaged reaction centers during illumination (9, 13, 33). Also, the size of the light-harvesting antenna (6) and the capacity of different protective mechanisms, e.g. photosynthesis (9, 14) and nonradiative dissipation of excitation energy (9), affect the rate of PSII photoinhibition (for review, see ref. 17).

Previously, we showed that high susceptibility of low-light-grown pumpkin (*Cucurbita pepo* L.) plants to photoinhibition cannot be attributed to a large size of the LHCII antenna per se but probably is mainly due to a poor capacity of the low-light-grown plants to repair photodamaged PSII centers (33). In the present paper, we give further evidence that the repair cycle of PSII indeed functions more slowly in low-light-grown pumpkin plants as compared with high-light-grown plants, and this is also seen as a poor capacity of the low-light leaves to degrade photodamaged D1 protein.

MATERIALS AND METHODS

Plant Material

Pumpkin (*Cucurbita pepo* L.) plants were grown in growth chambers at PPFD 80 and 1000 μmol m^{-2} s^{-1} with a 12-h light/dark rhythm at 22/18°C. Nearly fully expanded leaves of 3- to 5-week-old plants were used in the experiments.

High-Light Treatments

Leaves were detached, and the petioles were soaked in water or in CAP solution (1 mg/mL of H$_2$O) and incubated in darkness for 3 h. Side effects of CAP were checked in a set of control experiments in which another chloroplast translation inhibitor, lincomycin (Sigma, 808 units/mg, 1 mg/mL of H$_2$O), was used instead of CAP. The leaves were illuminated in a temperature-controlled growth chamber through a glass window at PPFD from 500 to 3000 μmol m^{-2} s^{-1} for 0.5 to 3 h at 20°C in saturating humidity. The temperature was continuously monitored during the treatments with a thermocouple attached to the nonilluminated side of a leaf. The CAP solution was protected from illumination. A 1200-

W HMI arc lamp served as a light source. To study the recovery from photoinhibition, the leaves were transferred to a growth chamber under dim light (50 μmol of photons m^{-2} s^{-1}) and 20°C, with the petioles in the same solution as during the high-light treatments.

Fluorescence Measurements

Fluorescence induction of leaf discs was measured with a pulse amplitude-modulated fluorometer (PAM 101, Heinz Walz, Effeltrich, Germany) at room temperature. After F_0 was measured, a 2-s pulse of saturating white light (PPFD 5500 μmol m^{-2} s^{-1}) was fired to obtain F_{MAX}. F_V is defined as F_{MAX} − F_0. When the actinic light was on, the pulse frequency of the PAM fluorometer was switched from 1.6 to 100 kHz. Before the fluorescence measurements, the leaf discs were allowed to adapt to the dark for 30 min on moist paper in a Petri dish. The fluorescence curves were analyzed with the FIP fluorescence software (Q_A-Data, Turku, Finland).

Isolation of Thylakoids and Electron Transfer Measurements

Thylakoids were isolated from control and illuminated leaves immediately after the treatments as described in ref. 4. Light-saturated electron transfer activity of PSII from H_2O to phenyl-p-benzoquinone (1 mM) was measured with a Hansatech oxygen electrode.

Chl

Chl was determined according to the procedure in ref. 2.

Chl-Protein Complexes

Chl-protein complexes were solubilized and electrophoretically separated as described earlier (33). The gels were scanned at 675 nm to determine the relative distribution of Chl between different Chl-protein complexes.

D1 Protein Quantification

Polypeptides of the thylakoid membranes were separated by denaturating SDS-PAGE (19) with a 12 to 22.5% gradient of acrylamide and 4 M urea in the separating gel. After electrophoretic separation, the polypeptides were transferred to an Immun-Lite membrane (Bio-Rad), and immunodetection of the D1 protein was performed with a Bio-Rad chemiluminescence kit. For D1 protein quantification, the immunoblots were scanned with an LKB laser densitometer. The D1 antibody was a generous gift from Professor I. Ohad. Total D1 was quantified as D1 plus D1*.

Control Experiments for Side Effects of CAP

We confirmed that the CAP treatment, if applied in the dark or in low light, did not affect the light-saturated rate of CO_2 fixation in detached leaves. However, light-saturated oxygen evolution measured in saturating CO_2 was 69 ± 8% of control (n = 5) after 24-h incubation of petioles in CAP solution in darkness. After leaves were treated similarly with lincomycin, light-saturated oxygen evolution was 96 ±

3% of that in control leaves, treated similarly but without inhibitors.

Lincomycin and CAP were also tested for their effect on decrease in F_V/F_{MAX} and degradation of the D1 protein induced by high-light treatment (PPFD 3000 μmol m^{-2} s^{-1}, 20°C) of isolated thylakoids. The thylakoid suspension (0.1 mg of Chl/mL) was illuminated for 45 min in a 10 mM sodium phosphate buffer solution, pH 7.4, containing 100 mM sucrose, 5 mM $MgCl_2$, and 20 mM NaCl. The concentration of both CAP and lincomycin was 1 mg/mL. F_{MAX} was continuously monitored with a PAM fluorometer. Photoinhibitory illumination was periodically switched off to check F_0, and after 45 min in high light, samples were collected for D1 quantification. We also found that lincomycin does not act as an autooxidizing electron acceptor of PSI in isolated thylakoids and participate in the production of active oxygen species, as suggested for CAP (28). The PSI electron transfer experiments were done with an oxygen electrode in saturating light in 40 mM phosphate buffer (pH 7.4), 1 mM NaCl, 0.6 mM NaN_3, 0.3 mM dichlorophenol-indophenol, 32 mM sodium ascorbate, 0.01 mM DCMU, and 1 mg/mL of lincomycin. The Chl concentration was 10 μg/mL.

Curve Fitting

The decrease in F_V/F_{MAX} during the high-light treatments in the presence of CAP was fitted to a first-order equation, weighting the data points individually according to their SE values. A model of two opposing first-order reactions, photoinhibition and recovery, was applied to the leaves treated in the absence of CAP, using the equation (35)

$$F = \frac{k_{REC} + k_{PI}e^{-(k_{PI} + k_{REC})t}}{k_{PI} + k_{REC}},$$

where $F = F_V/F_{MAX}$ expressed as a fraction of the control value, k_{PI} = rate constant for photoinhibition, k_{REC} = rate constant for the recovery, and t = time.

To determine the rate constant for concurrent recovery, k_{REC}, we assumed that the substrate for the recovery reaction is the product of photoinhibition and that there are no photoinhibited centers in untreated leaves. k_{PI} was adapted from the treatment at the same PPFD in the presence of CAP. The Fig.P software (Biosoft, Cambridge, UK) was used for curve fitting.

RESULTS

Illumination of high-light leaves at PPFD from 500 to 2000 μmol m^{-2} s^{-1} induced only slight inhibition of PSII, demonstrated as a small (up to 20%) decrease of F_V/F_{MAX} in the course of illumination (Fig. 1, A–C). Only when the leaves were illuminated at 3000 μmol m^{-2} s^{-1} (Fig. 1D) did PSII become severely photoinhibited (60% reduction in F_V/F_{MAX} in 3 h). Low-light leaves were much more susceptible, showing severe photoinhibition already at 1000 μmol m^{-2} s^{-1} (Fig. 2). The decrease in F_V/F_{MAX} during illumination in the absence of CAP could not be fitted to a first-order equation but showed equilibration to a certain level of inhibition (Figs. 1 and 2). Equilibration is expected if a first-order recovery reaction is counteracting a first-order inhibition reaction dur-
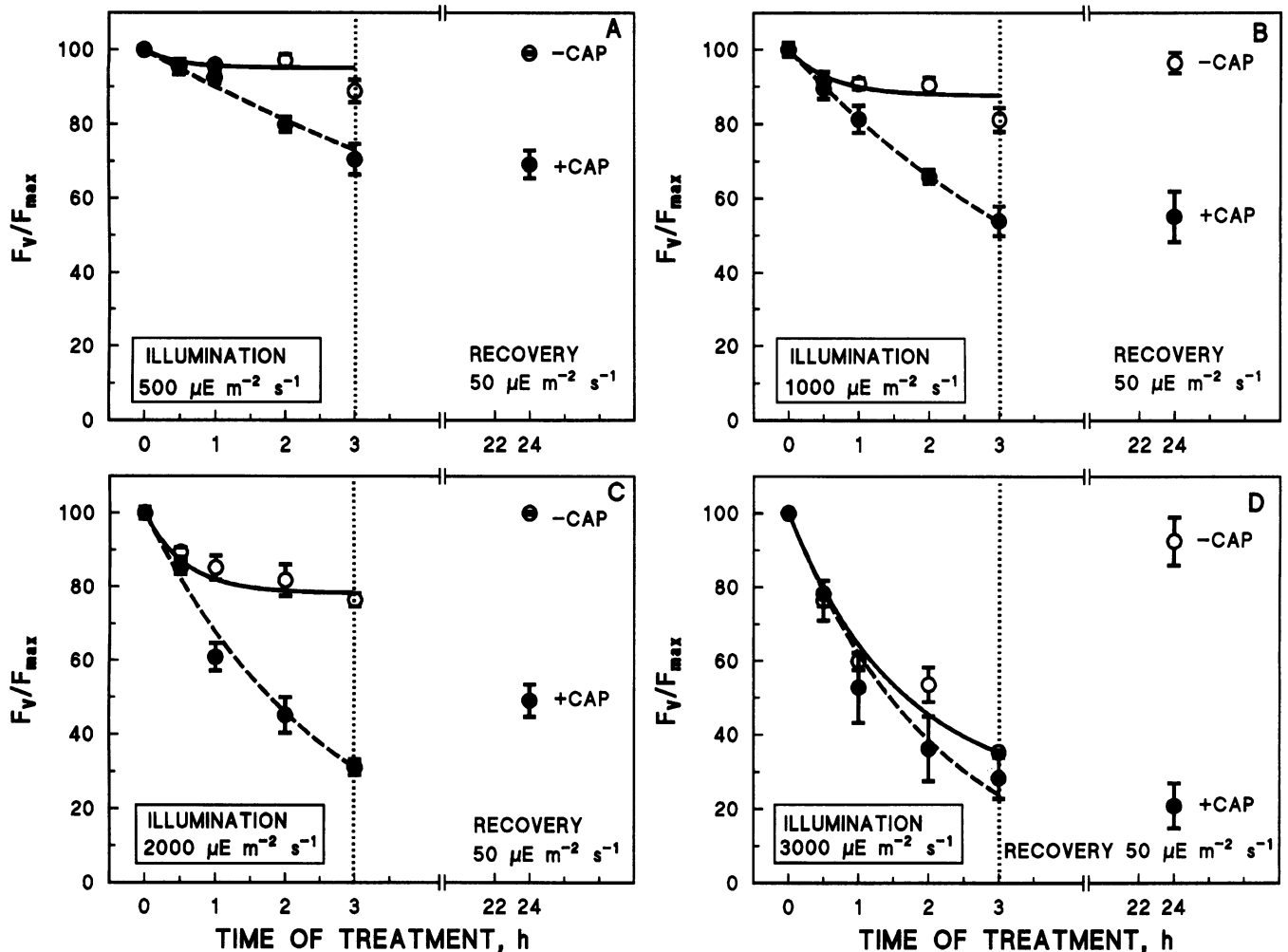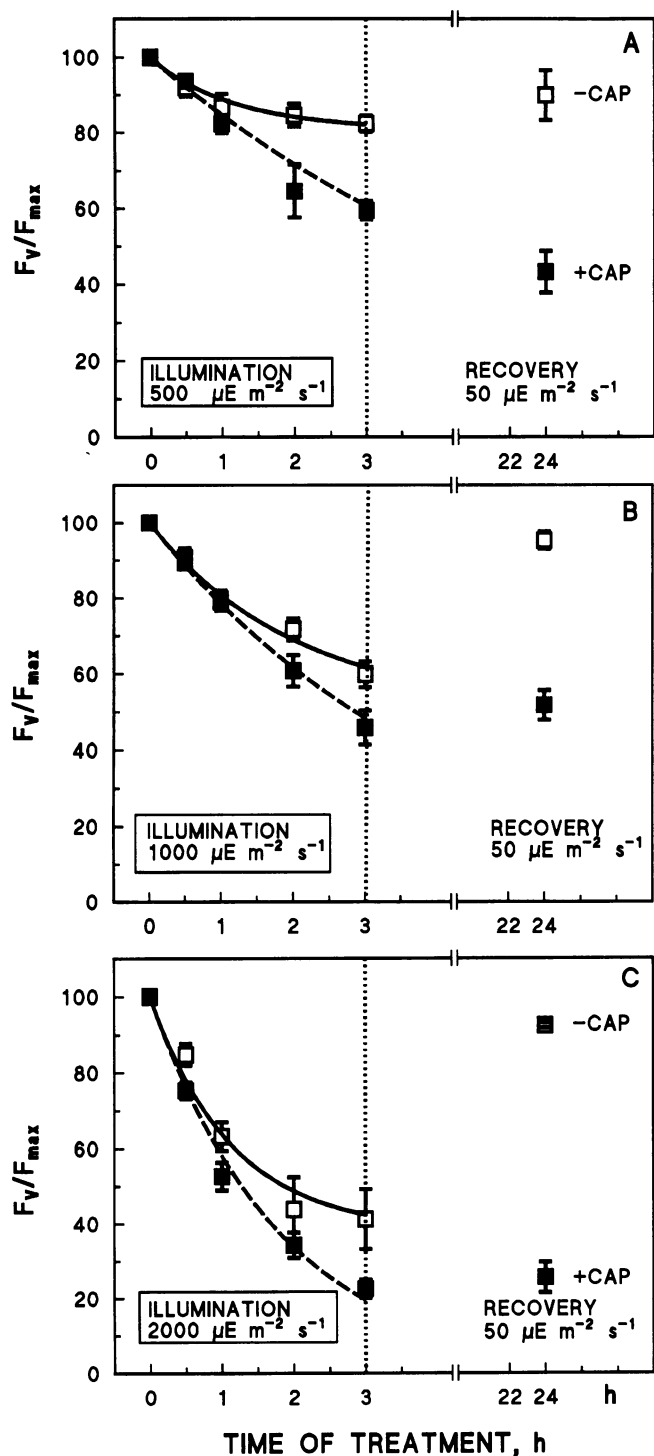
Figure 1. Photoinhibition of PSII (estimated from decrease in F_V/F_{MAX}) in high-light pumpkin leaves during illumination at different PPFD at 20°C. Subsequent recovery was in dim light. The leaves were illuminated either in the absence (O) or presence (●) of CAP. The results are means of three to six independent experiments and are expressed as percentages of control. Bars represent SE. Control F_V/F_{MAX} was 0.85. The curves represent fits to first-order reaction kinetics in CAP-treated leaves. The curves of control leaves (without CAP) represent fits to two opposing first-order reaction kinetics, those of photoinhibition and concurrent recovery. The k_{PI} was obtained from the corresponding curve of CAP-treated material. In all cases, the goodness of the fit was within 95% of the expected limits for a correct model of the data.

ing the high-light treatment. When chloroplast protein synthesis was blocked during illumination with CAP, the decrease in F_V/F_{MAX} was enhanced and proceeded now with first-order kinetics (Figs. 1 and 2), the rate of which depended linearly on the PPFD of the treatment (Fig. 3). It could be argued that the decrease in F_V/F_{MAX} was partially due to photoprotective processes. However, the leaf discs were dark adapted for 30 min before the fluorescence measurements, and unpublished data from our laboratory indicates that, in pumpkin leaves, this is long enough to allow for relaxation of a light-induced decrease in F_V/F_{MAX} that is not accompanied by a decrease in light-saturated PSII activity. Assignment of the decrease in F_V/F_{MAX} to photoinhibition is also supported by the finding that there was no statistically significant partial recovery of F_V/F_{MAX} after the treatments in the presence of protein synthesis inhibitors (Figs. 1 and 2).

In the presence of CAP, the difference in the susceptibility

to photoinhibition between high- and low-light leaves was also greatly diminished (Figs. 1 and 2). Although the k_{PI} values were somewhat higher in low-light leaves at all measured PPFD, a much more significant difference was found in the estimated k_{REC} (Fig. 3). This suggests that the capacity of chloroplast protein synthesis and the rate of D1 turnover largely govern the difference in susceptibility of high-light and low-light leaves to strong illumination. Our data do not prove that the concurrent recovery is a first-order process. However, the data fit nicely in such a model, and a first-order concurrent recovery was also recently found to operate in *Synechococcus* during photoinhibition (35).

High CAP concentrations may have serious side effects during illumination of leaves or chloroplasts (28). To ensure that the enhancement of photoinhibition in the presence of CAP was due to inhibition of protein synthesis, we compared CAP with lincomycin, which is another efficient translation

Figure 2. Photoinhibition of PSII in low-light pumpkin leaves. The leaves were illuminated in the absence (□) or presence (■) of CAP. See the legend of Figure 1 for all other details.

inhibitor in chloroplasts (16). Photoinhibition and recovery experiments with high-light leaves at 20°C, PPFD 1500 μmol m^{-2} s^{-1}, showed that lincomycin and CAP had the same effect on photoinhibition measured either as a decrease in F_V/F_{MAX} (Fig. 4A) or as a decrease in PSII activity of thylakoids isolated from treated leaves (data not shown). Both inhibitors also enhanced the loss of the D1 protein during photoinhibition in vivo quite similarly, although a slight additional effect of CAP cannot be ruled out (Fig. 4B). Both antibiotics inhibited recovery from photoinhibition (Fig. 4A). When isolated thylakoids were illuminated in vitro, lincomycin did not accelerate the decrease in F_V/F_{MAX}, and CAP had only a neligible effect (data not shown). D1 degradation was not significantly enhanced in isolated thylakoids when illuminated in vitro in the presence of CAP (1 mg/mL) or lincomycin (1 mg/mL), as compared with illumination without these compounds (Fig. 4C). Because the effects of CAP and lincomycin on PSII photoinhibition and degradation of the D1 protein were very similar in both intact leaves and isolated thylakoids, we conclude that the effects of CAP in the photoinhibition experiments with pumpkin leaves presented in this communication are indeed due to the inhibition of chloroplast protein synthesis.

Because these data indicated that the repair of photodamaged PSII may be a crucial factor in the differential susceptibility of high- and low-light-grown pumpkin leaves to photoinhibition, we focused next on the removal and degradation of photodamaged D1, one of the first phases in the complicated repair cycle of PSII (1, 24, 25).

High- and low-light leaves were illuminated in the presence of CAP to induce 50 to 80% photoinhibition of PSII electron transfer (H$_2$O to phenyl-p-benzoquinone) measured from thylakoids isolated from both kinds of leaves after the illumination (Fig. 5). The quantity of the D1 protein in the
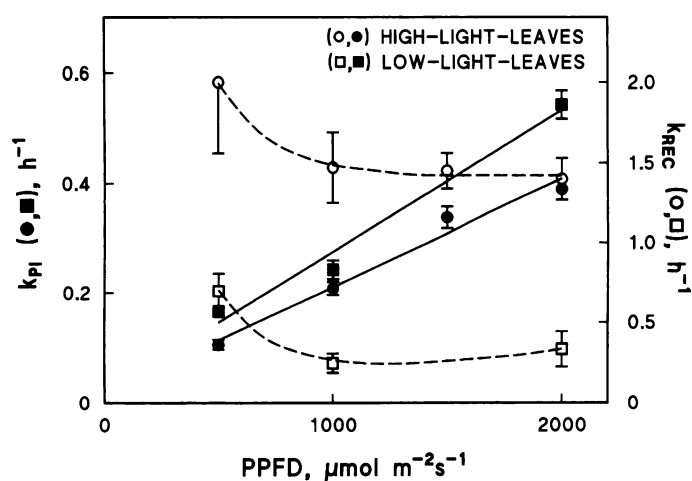
Figure 3. k_{PI} (●, ■) and k_{REC} (O, □) in high- and low-light-grown pumpkin leaves, respectively, as a function of PPFD. k_{PI} was obtained from leaves treated in the presence of CAP, and k_{REC} was calculated by fitting the decrease in F_V/F_{MAX} in leaves treated in the absence of CAP to a model of two opposing first-order reactions and adapting the k_{PI} from the CAP-treated leaves. The fitted curves are shown in Figures 1, 2, and 4.
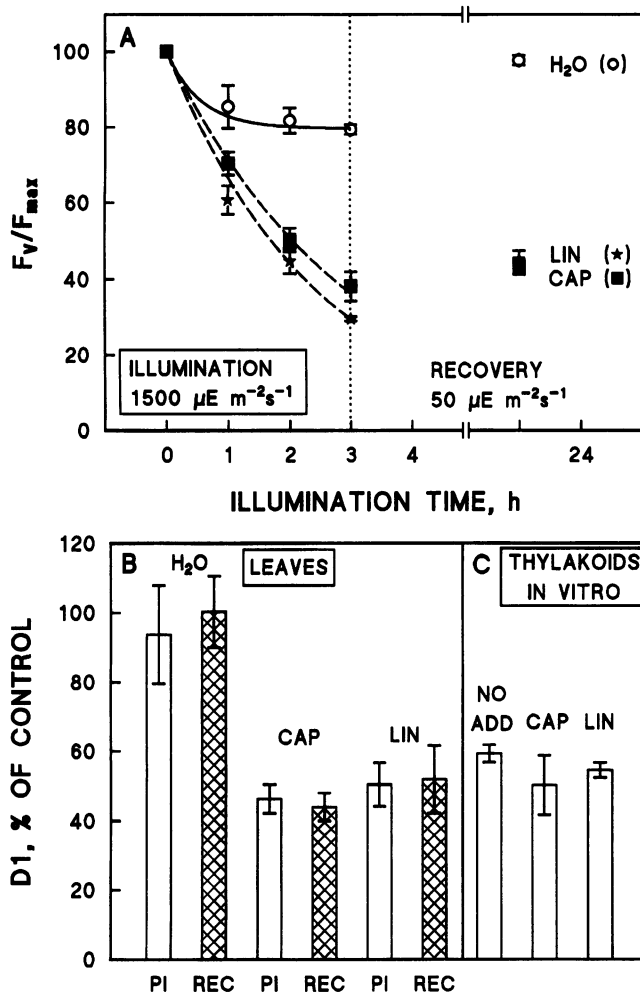
Figure 4. Comparison of the effects of CAP and lincomycin (LIN) on decrease of F_V/F_{MAX} (A) and loss of D1 protein (B) during high-light treatment of intact leaves and on loss of D1 during photoinhibition of isolated thylakoids in vitro (C). In A and B, high-light leaves were illuminated at the PPFD of 1500 μmol m^{-2} s^{-1} for 3 h with their petioles in water (O), CAP (■), or LIN (*) and allowed to recover at the PPFD of 50 μmol m^{-2} s^{-1}, 20°C, for 22 h after the treatment with the petioles in the same solution. During the high-light treatment and after the recovery period, leaf discs were punched for F_V/F_{MAX} determination. The control F_V/F_{MAX} value was 0.86 ± 0.002 for the three treatments. In B, the D1 protein was quantitated by immonoblotting from thylakoids isolated from control leaves, leaves illuminated with high-light for 3 h (PI), and after a subsequent 22-h recovery period in low light (REC). In C, isolated thylakoids were illuminated for 45 min at the PPFD of 3000 μmol m^{-2} s^{-1} at 20°C in the absence or presence of CAP or LIN. The data are means ± SE of three independent experiments and are expressed as percentages of the control.

same thylakoid preparations was followed by immunoblotting. Figure 5 shows that the loss of the D1 protein, measured from thylakoids isolated from treated leaves, is severely retarded in low-light leaves compared with high-light leaves. This suggests that the susceptibility of low-light leaves to strong illumination and their poor ability to recover from

photoinhibition (33) largely derive from the low capacity of these leaves for degradation of photodamaged D1.

We have previously reported that in intact pumpkin leaves net loss of the D1 protein from thylakoid membranes can be demonstrated only if photoinhibition of PSII is induced in the presence of an inhibitor of chloroplast protein synthesis (15). The D1 protein is modified during illumination, and the slightly slower electrophoretic mobility of this D1* allows separation from the original D1 protein (15). When low-light leaves were illuminated in the presence of a chloroplast protein synthesis inhibitor, most D1 was modified to D1* before significant loss of the protein from the thylakoid membranes could be detected (Fig. 6). However, D1 degradation was initiated during the high-light treatment and continued for several hours after the leaves were transferred to dim light (recovery conditions) (Fig. 7A). In high-light leaves, degradation of the D1 protein in the presence of CAP more readily followed the course of photoinhibition of PSII electron transport during illumination (Fig. 7B).

Figure 8 shows electron micrographs of chloroplasts of high- and low-light-grown pumpkin leaves. Low-light leaves possess chloroplasts with much more intense thylakoid stacking than the high-light leaves. Estimation of the LHCII antenna size of PSII from the Chl-protein composition of the thylakoid membranes revealed that the ratio of LHCII complexes to the internal antenna complexes of PSII was 2-fold larger in low- than in high-light thylakoids.

DISCUSSION

Low-light-grown plants have frequently (9, 14, 30) been reported to be more sensitive to photoinhibition than high-
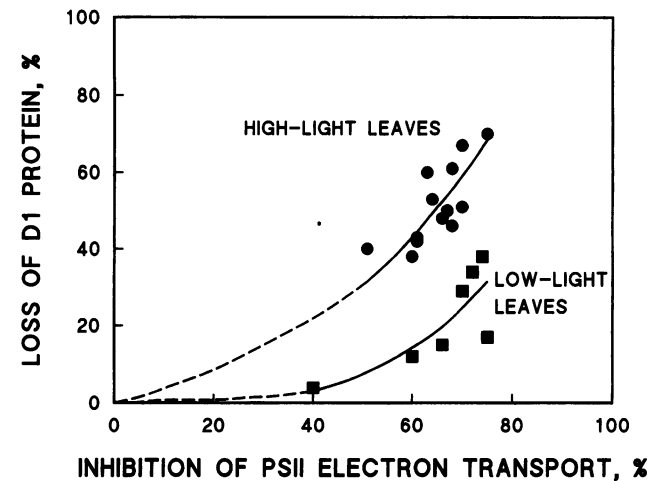
Figure 5. Comparison of photoinhibition of PSII electron transport (H$_2$O to phenyl-p-benzoquinone) and loss of the D1 protein in high-light (●) and low-light (O) pumpkin leaves. High-light leaves were illuminated at PPFDs of 1500 (3 h) and 3000 μmol m^{-2} s^{-1} (1.5–3 h) and low-light leaves at 1500 μmol m^{-2} s^{-1} (1–3 h) in the presence of CAP. The light-saturated PSII activity was measured and the D1 protein was quantified by immunoblotting from thylakoids isolated from control and illuminated leaves. Both D1 and D1* bands are included in the amount of the D1 protein. All values are expressed as percentages of the control. Each data point represents one experiment.
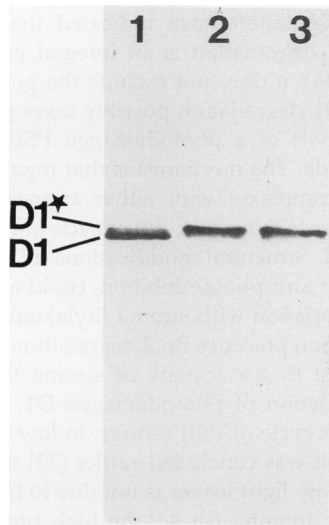
Figure 6. An immunoblot demonstrating the modification (D1 to D1*) but only slow degradation of the D1 protein during photoinhibitory illumination of low-light pumpkin leaves in the presence of CAP. Leaves were illuminated at a PPFD of 1500 μmol m^{-2} s^{-1} for 2 and 3 h at 20°C, inducing 65 and 72% inhibition of light-saturated PSII electron transport, respectively. Thylakoids equivalent to 3 μg of Chl were applied to each well of the gel. The levels of D1 and D1* in nonilluminated leaves (lane 1) and in leaves illuminated for 2 h (lane 2) and 3 h (lane 3) are shown.
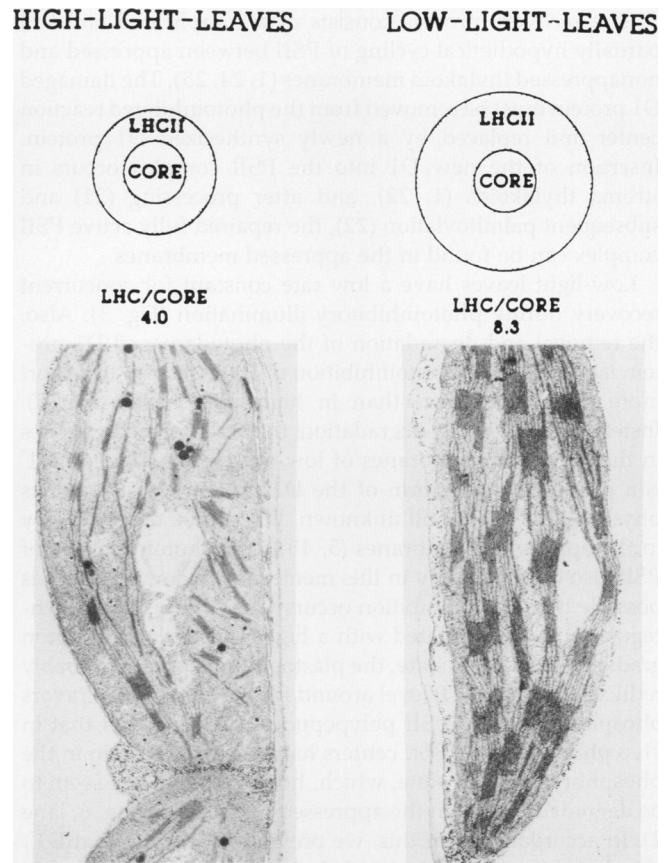
Figure 8. Electron micrographs of chloroplasts of palisade parenchyma cells of high- and low-light leaves. The numbers indicate the relative size of the light-harvesting antenna of PSII in each chloroplast type as a ratio of total LHCII to the internal antennae of PSII core.
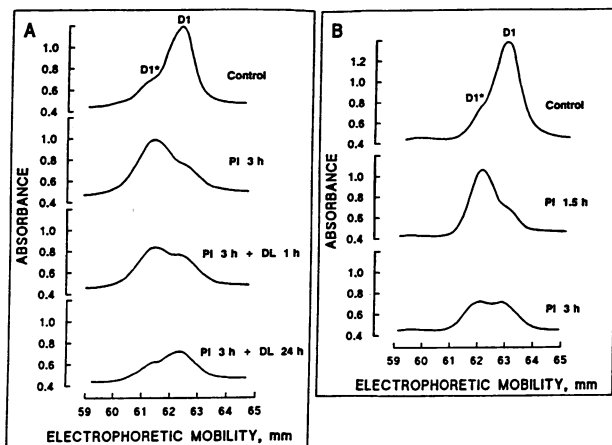
Figure 7. Laser densitograms of immunoblots of the D1 protein indicating slower degradation in low-light leaves (A) than in high-light leaves (B) during illumination in the presence of CAP. High- and low-light leaves were illuminated for 3 h to induce approximately 70% photoinhibition of light-saturated PSII electron transport activity. In high-light leaves, D1 was already mostly degraded during the illumination (B), whereas in low-light leaves the modified D1 protein (D1*) accumulated and was only slightly degraded during illumination. The degradation occurred mainly after transfer of the leaves to dim light. Thylakoids equivalent to 3 μg of Chl (A) and 1.5 μg of Chl (B) were applied to each well. PI, Photoinhibitory treatment; DL, subsequent incubation in dim light.

light-grown plants. Generally, the sensitivity to photoinhibition is thought to be governed by various factors such as the size of the light-harvesting antenna of PSII, the capacity to repair photoinhibited PSII during illumination, and the efficiency of various mechanisms that dissipate excitation energy harmlessly (for review, see ref. 17). Photosynthesis can also be regarded as a protective mechanism against the adverse effects of high light.

Our present experiments show that when chloroplast protein synthesis is inhibited during illumination the high-light grown pumpkin leaves become almost as susceptible to illumination as the low-light leaves (Fig. 3). This supports our earlier conclusion from in vitro experiments (33) that the size of the light-harvesting antenna does not much affect the rate of PSII photoinhibition. More efficient photoprotective mechanisms and faster photosynthesis in the high-light-grown plants probably also contribute to the persisting difference in susceptibility between high- and low-light plants in the presence of CAP (Fig. 3).

The resistance of high-light leaves to photoinhibition can be attributed largely to efficient repair of photodamaged PSII centers during illumination, as can be concluded from Figure

3. The recovery process consists of a complicated and still partially hypothetical cycling of PSII between appressed and nonappressed thylakoid membranes (1, 24, 25). The damaged D1 protein must be removed from the photoinhibited reaction center and replaced by a newly synthesized D1 protein. Insertion of the new D1 into the PSII complex occurs in stroma thylakoids (1, 22), and after processing (21) and subsequent palmitoylation (22), the repaired fully active PSII complex can be found in the appressed membranes.

Low-light leaves have a low rate constant for concurrent recovery during photoinhibitory illumination (Fig. 3). Also, the removal and degradation of the photodamaged D1 protein lag behind the photoinhibition of PSII electron transport more severely in low- than in high-light leaves (Fig. 5). Instead of efficient D1 degradation, the D1* form accumulates in the thylakoid membranes of low-light leaves (Fig. 6). D1* is a phosphorylated form of the D1 protein (3, 10), but its physiological role is still unknown. D1* can be detected only in the appressed membranes (5, 15), and photoinhibition of PSII also occurs mainly in this membrane region (7, 20). It is possible that photoinhibition occurs when PSII is in a down-regulated state associated with a high transthylakoid proton gradient (29). In this state, the plastoquinone pool is probably reduced, and the ATP level around PSII is high, which favors phosphorylation of PSII polypeptides. This suggests that in vivo photoinhibited PSII centers have their D1 protein in the phosphorylated D1* state, which, however, does not seem to be degraded readily in the appressed membranes (Fig. 6, lane 2). In accordance with this, we previously showed that D1*, if induced by ATP in isolated thylakoids, is less susceptible to degradation than the original D1 protein (3).

Comparing the capacity of high- and low-light leaves to degrade the D1 protein of photoinhibited PSII centers (Fig. 5), on the one hand, and the thylakoid organization of these chloroplasts, on the other (Fig. 8), leads us to suggest that stroma thylakoids are important in D1 protein degradation. Moreover, results of the photoinhibition experiments performed without inhibitors of chloroplast protein synthesis suggest that D1 protein degradation and insertion of newly synthesized D1 protein to the reaction center complex might be closely synchronized, because almost no net loss of the D1 protein (8, 15, 26) from the thylakoid membranes can be observed in spite of severe photoinhibition of PSII. However, our data do not exclude the possibility that degradation and synthesis of the D1 protein occur with similar rates but independently of each other. In pumpkin leaves, net loss of the D1 protein in the absence of CAP occurs only if the high-light treatment of the leaves is severe enough to prevent full recovery after transfer to dim light (15). Such severe treatments also lead to Chl bleaching, which becomes evident several hours after the treatment. To our knowledge, recovery from photoinhibition after a large net loss of the immunologically detected amount of the D1 protein has never been demonstrated. The fact that the in vivo amount of the D1 protein stays fairly constant over a wide range of light intensities in the absence of protein synthesis inhibitors (31) not only indicates that D1 synthesis can normally match the rate of its degradation but may also suggest that degradation and synthesis of the D1 protein are interregulated.

Although in vitro experiments on PSII photoinhibition and D1 protein degradation have indicated that the protease involved in D1 degradation is an integral part of the PSII core complex (34), it does not exclude the possibility that in vivo the final D1 degradation possibly takes place during or after the transport of a photodamaged PSII center to the stroma thylakoids. The mechanisms that regulate the association of PSII complexes with either appressed or nonappressed membranes during the repair cycle of PSII still remain to be elucidated. Structural modifications in PSII, caused by phosphorylation and photoinhibition, could also function as a signal for association with stroma thylakoids, and perhaps dephosphorylation precedes final degradation of the D1 protein. We suggest that a scarcity of stroma thylakoids may limit the degradation of photodamaged D1, and therefore, the whole repair cycle of PSII centers, in low-light leaves.

Even though it was concluded earlier (33) that faster photoinhibition in low-light leaves is not due to large size of the light-harvesting antenna per se, the high proportion of the LHCII complex induces extensive thylakoid stacking, which may indirectly influence the repair process of photodamaged PSII centers. The strategy of low-light plants is to accumulate light-harvesting Chl to ensure efficient photosynthesis when light is a limiting factor. Under conditions of occasional high-light exposure, the PSII centers may become easily photoinhibited because the concurrent recovery is slow. However, the photoinhibited PSII centers remain structurally intact in the appressed membranes and at that stage are efficient in trapping the excitation energy but dissipate it nonphotochemically (7). By this means, the low-light leaves may maximize avoidance of totally irreversible photooxidative damage to the thylakoid membranes without maintaining an energetically expensive, fast repair cycle of PSII.

ACKNOWLEDGMENTS

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