Resistance to Low Temperature Photoinhibition Is Not Associated with Isolated Thylakoid Membranes of Winter Rye¹

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ABSTRACT

In vivo measurements of chlorophyll a fluorescence indicate that cold-hardened winter rye (Secale cereale L. cv Musketeer) develops a resistance to low temperature-induced photoinhibition compared with nonhardened rye. After 7.2 hours at 5°C and 1550 micromoles per square meter per second, the ratio of variable fluorescence/maximum fluorescence was depressed by only 23% in cold-hardened rye compared with 46% in nonhardened rye. We have tested the hypothesis that the principal site of this resistance to photoinhibition resides at the level of rye thylakoid membranes. Thylakoids were isolated from cold-hardened and nonhardened rye and exposed to high irradiance (1000–2600 micromoles per square meter per second) at either 5 or 20°C. The photoinhibitory response measured by room temperature fluorescence induction, photosystem II electron transport, photoacoustic spectroscopy, or [¹⁴C]atrazine binding indicates that the differential resistance to low temperature-induced photoinhibition in vivo is not observed in isolated thylakoids. Similar results were obtained whether isolated rye thylakoids were photoinhibited or thylakoids were isolated from rye leaves preexposed to a photoinhibitory treatment. Thus, we conclude that increased resistance to low temperature-induced photoinhibition is not a property of thylakoid membranes but is associated with a higher level of cellular organization.

The phenomenon of photoinhibition was described first by Kok and Businger (16) in green algae and subsequently has been observed in many oxygenic plant species when exposed to light conditions that are in excess of the photon requirement for photosynthesis (26). Susceptibility to photoinhibition is manifested as a reversible reduction in the quantum yield and light saturated rates of CO₂ uptake or CO₂-dependent O₂ evolution or as a decrease in the room temperature fluorescence ratio of F₅/Fₐ (4).

The primary site of photoinhibitory damage has been shown to be localized to PSII in vivo and in vitro (2, 17, 26). However, the precise site within PSII that initially becomes photodamaged and the mechanism by which this damage occurs is still controversial (2, 17). For example, it has been proposed that the primary site of photoinhibitory dysfunction is the inactivation of the PSII reaction center itself or the donor side of PSII (2, 3, 6, 8, 9, 35, 39). This inactivation occurs with no apparent loss in herbicide binding capacity to the QA binding site of the 32 kD polypeptide of PSII. Most of these data have been obtained through in vitro photoinhibitory studies of isolated thylakoid membranes. In contrast, there is evidence that the primary site of damage during photoinhibition of PSII is the destruction and increased turnover of the 32 kD polypeptide of PSII (20, 27, 28). This leads to a decreased ability to bind herbicides at the QA binding site on the acceptor side of PSII. Much of this work is the result of photoinhibitory studies employing Chlamydomonas reinhardii. Thus, whether the primary site of photoinhibitory damage is at the level of the PSII reaction center itself or at the level of the QA binding site cannot be ascertained unequivocally at this time.

The phenomenon of photoinhibition measured in vivo appears to be prevalent even under moderate light conditions when plants are subjected to environmental stresses such as chilling (5, 11, 23–26, 30, 32, 34, 37) and freezing (17, 24, 30, 33). This light-dependent reduction in photosynthetic efficiency in vivo is typically reversible and is not always associated with photodamage to PSII. Recently, photoinhibitory fluorescence quenching through the thermal deactivation of excited state pigments has been shown to be an important mechanism to prevent photodamage to PSII (10). Demmig and coworkers (10) have shown that photoinhibitory induced

¹ Abbreviations: Fᵥ, variable fluorescence; Fₐ, maximum fluorescence with all PSII traps closed; F₀, minimal fluorescence with all PSII traps open; RH, cold-hardened rye; RNH, nonhardened rye; LHCII, light-harvesting Chl a/b protein complex associated with PSII; DCPIP, 2,6-dichlorophenolindophenol; MV, methylviologen; PAS, photoacoustic spectroscopy; φₐ, relative energy storage yield; QA, second electron-accepting plastoquinone of PSII.

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increases in zeaxanthin content are correlated with greater protection of PSII through increased nonradiative decay of excitation energy. Schöner and Krause (30) concluded that changes in the xanthophyll levels of cold-hardened spinach thylakoids may be important in protecting cold-hardened spinach against photoinhibition. They also reported higher levels of scavengers of active O₂ species in cold-hardened than in nonhardened spinach plants, which could be implicated in the plant's resistance to photoinhibition at chilling temperatures.

Cold-resistant plant species such as winter rye (Secale cereale L. cv Musketeer) and spinach can be photoinhibited at low temperatures if given sufficiently high irradiance. However, it appears that growth and development at low temperature induces a unique resistance to the light-dependent reduction in Fv/Fm and in the apparent quantum yield for O₂ evolution when measured in vivo in winter rye (25) and spinach (5, 30, 32, 33, 34). Examination of the recovery kinetics from photoinhibition for RH and RNH plants indicates that resistance cannot be accounted for by faster recovery rates (25). Rye leaves must develop at the low temperature to exhibit this increased resistance to low temperature photoinhibition (25). Similar observations have been made in cold-hardened and nonhardened spinach (S.R. Boese and N.P.A. Huner, unpublished observation) and wheat (Hurry and N.P.A. Huner, unpublished observation).

Rye thylakoids have been shown to be modified during growth and development at low temperatures. Huner et al. (14, 15, 19) have reported that development at cold-hardening temperatures results in specific organizational changes in the LHCII. In situ electron microscopy and freeze fracture indicated that low temperature development results in smaller granal stacks and a decreased PSII-LHCII particle size (13.6 nm) compared with RNH thylakoids (16.0 nm) (12). In vitro, it has been shown that RH LHCl is stabilized primarily in its monomeric form, whereas RNH LHCl is stabilized in the oligomeric form (14, 15). These data have been supported by differential scanning calorimetry and 77 K fluorescence of isolated rye thylakoids (14). However, the development of rye leaves at cold-hardening temperatures did not induce any change in the ratio of carotenoid/Chl (12).

We have been interested in elucidating the mechanism of cold-hardening-induced resistance to photoinhibition. Because PSII of thylakoid membranes is the primary site of a photoinhibitory-induced decrease in photosynthetic efficiency as measured by in vivo fluorescence (Fv/Fm) or apparent quantum yield for O₂ evolution, it follows that a resistance to a photoinhibitory decrease in Fv/Fm may be a property of rye thylakoid membranes. If these modifications in PSII-LHCII units in rye thylakoid membrane organization observed during cold hardening impart resistance to photoinhibition, isolated RH thylakoids should exhibit more resistance to the photoinhibitory-induced decrease in PSII efficiency than RNH thylakoids. In this report, we test this hypothesis by an examination of the susceptibility of isolated thylakoids from cold-hardened and nonhardened rye to photoinhibition using Chl fluorescence, in vitro electron transport activity, photoacoustic spectroscopy, and [¹⁴C]atrazine binding.

### MATERIALS AND METHODS

#### Plant Material

Seeds of winter rye (Secale cereale L. cv Musketeer) were sown in vermiculite and germinated in a growth cabinet at 20/16°C (day/night) under a photoperiod of 16 h and a PPFD of 250 μmol m⁻² s⁻¹. After 7 d, seedlings were either kept at 20/16°C (day/night) for an additional 2 weeks or transferred to 5/5°C (day/night) for 8 weeks with all other conditions held constant. According to Krol et al. (18), these cold- and warm-grown rye plants are of comparable physiological ages.

#### Thylakoid Isolation

Thylakoids were isolated from fully expanded rye leaves by grinding in a Waring blender (2 × 5 s bursts) in the following buffer: 0.4 M sorbitol, 50 mM Tricine (pH 7.8), 10 mM NaCl cooled to 0°C. The homogenate was filtered through two layers of Miracloth and centrifuged at 3000g for 2 min at 4°C. The pellet was washed once in buffer containing 0.1 M sorbitol, 50 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM MgCl₂, and 1 mM NH₄Cl and kept on ice in the dark until used. Thylakoids were resuspended in the presence of uncouplers to eliminate the effect of nonphotochemical quenching caused by the buildup of a light-dependent trans-thylakoid pH gradient. However, preliminary study of photoinhibition of rye thylakoids in the absence of uncouplers showed the same trends as those described in this paper. Freshly prepared thylakoids were used in all cases.

Chl concentrations were determined according to Arnon (1). Thylakoid suspensions were diluted with resuspension buffer to a Chl concentration of 150 μg mL⁻¹.

#### Photosynthetic Electron Transport Activity

PSII activity (H₂O → DCPIP) was measured in the resuspension buffer containing 12 μg Chl mL⁻¹ and 60 μM DCPIP. The reduction of DCPIP was followed in a Unicam SP1800 spectrophotometer at 590 nm in a jacketed cuvette thermostatically regulated at 25°C. A light source, filtered through Cinemoid ruby No. 14 and an orange No. 5 (Strand Electric) filters, was used to excite the thylakoid sample. PSII activities were corrected for dark decay of thylakoids at room temperature as a function of time.

PSI activity (ascorbate/DCPIP → MV) was measured in the resuspension buffer containing 12 μg Chl mL⁻¹ as well as 20 μM DCMU, 5 mM NH₄Cl, 200 μM MV, 60 μM DCPIP, 1 mM NaN₃, and 61 μg mL⁻¹ of superoxide dismutase. The reduction of MV was followed polarigraphically at 20°C with a Hansatech aqueous phase O₂ electrode. The reaction was started by turning on the light. PPFD was attenuated with neutral density filters.

#### Room Temperature Fluorescence Induction

Room temperature fluorescence induction curves were obtained using a PAM 101 chlorophyll fluorometer (Walz) that has been described in detail (31). PPFD of the modulated beam at 650 nm was 0.12 m² s⁻¹ and the frequencies used
were 1.6 kHz for F₀ measurements and 100 kHz when used in conjunction with a pulse of saturating white light (2750 μmol m⁻² s⁻¹). The duration of the flashes was 400 ms with 5 s between each pulse. The fluorescence signals were recorded on an X-Y recorder Omnigraphic 2000 (Houston Instruments). The samples were diluted to 20 μg Chl mL⁻¹. F, and Fₘ were corrected for dark decay of thylakoids at room temperature as a function of time.

**Photoacoustic Measurements**

PAS measurements were performed as described in detail by Carpentier *et al.* (7). The fluorescence rate of the modulated measuring light beam of 680 nm was varied between 0.16 and 4.15 W m⁻² at a frequency of 35 Hz. The nonmodulated saturating light beam had a fluence rate of 186 W m⁻². All measurements were made at room temperature. The thylakoids (240 μg Chl total) were aspirated through a nitrocellulose filter (Millipore Corp., AA type, 25 mm diameter, 0.8 μm pore size) that was then cut to a final diameter of 15 mm. A new sample was prepared for each photoacoustic measurement. The φ', was calculated from the difference between the intensity of the PAS signal in the presence and in the absence of the nonmodulated saturating beam. Correction factors were applied to φ', to take into account dark decay of thylakoids at room temperature as a function of time.

**Atrazine Binding**

Atrazine binding was carried out according to Tischer and Strotmann (36). Resuspended thylakoids diluted to 48 μg Chl mL⁻¹ were pipetted in an Eppendorf tube and 20 μL of [ethyl-1-¹⁴C]atrazine (925 kBq μmol⁻¹) were added for a final concentration of 1 μM. The total reaction volume was 1 mL. Thylakoid membranes were incubated for 10 min at room temperature and low light (2 μmol m⁻² s⁻¹), then centrifuged for 3 min at 16000g at 5°C. The supernatant (0.7 mL) was added to 10 mL of AQUASOL-2 (DuPont) and radioactivity determined in a Beckman scintillation counter (model LS6000IC). Control samples without thylakoids were processed in the same way. The amount of thylakoid-bound atrazine was calculated from the difference between controls and the corresponding thylakoid samples. An initial kinetic binding study was performed to determine the optimal concentration range required to saturate the high affinity binding sites. Double reciprocal plots of the binding data indicated that atrazine binding was biphasic as expected (36) and that 1 μM atrazine was sufficient to saturate the high affinity binding sites. The number of binding sites for atrazine in RNH and RH control thylakoids was not significantly different and was calculated to be 4.5 ± 0.3 and 6.3 ± 1.7 nmol of atrazine mg Chl⁻¹, respectively. Thus, for rye thylakoids, there is about 1 atrazine binding site for every 212 Chl molecules. These values represented control levels prior to photoinhibition and were used as an estimate of 100% binding capacity. The reduction in the number of atrazine binding sites after a photoinhibitory treatment was calculated relative to these control values.

**Photoinhibitory Treatment of Thylakoids**

Isolated rye thylakoids were exposed to white light from a Fiber-Lite light source (model 170-D) directed to the sample via an optic fiber bundle. The samples were stirred continuously during the treatment. The treatments were performed at either room temperature or in a cold room at a PPFD of 2600 μmol m⁻² s⁻¹ at the center of the vial. The temperature of the sample was measured at the beginning and at the end of each photoinhibitory treatment and varied from 5 to 7°C at the low temperature and from 20 to 25°C at the high temperature. Control samples were kept at either room temperature or 5°C in the dark. For Chl fluorescence measurements, all samples were dark adapted for 10 min after the photoinhibitory treatment prior to measurements of Chl fluorescence. For PSI, PSII, and PAS measurements, the samples were measured immediately after the photoinhibitory treatment.

**In Vivo Photoinhibitory Treatments**

Rye segments were placed on moist filter paper and exposed to a PPFD of 1500 to 1550 μmol m⁻² s⁻¹ using high pressure sodium vapor lamps. The samples were shielded from the light by a 10 cm heat filter that consisted of continuously flowing water. The air above the samples was circulated using two small fans. Sample temperatures remained between 5 and 7°C under these conditions. Leaf sections were dark adapted at room temperature for 30 min before Chl a fluorescence was measured and then thylakoids were isolated from the treated leaf segments. Fluorescence was measured with a Plant Stress Meter Mark II (Bio Monitor, Sweden) on leaf segments as well as on thylakoids isolated from the leaf segments.

All PPFD were measured with a Li-Cor light meter (model LI-185A) from the end of the optic fiber bundles. Each experiment presented here was repeated at least three times for electron transport and PAS measurements and four times for the Chl fluorescence measurements and atrazine binding studies. All data are presented as the average ± se. Where error bars are not obvious in the figures, the se was the same size as the symbol.

**RESULTS**

The *in vivo* Chl a fluorescence results for rye leaf segments illustrated in Figure 1 indicate that rye leaves developed under cold-hardening conditions develop a resistance to low temperature-induced photoinhibition compared with rye leaves developed under nonhardening conditions. After 7.2 h at 5°C and an irradiance of 1550 μmol m⁻² s⁻¹, the Fₘ/Fₘ was depressed by 46% in RNH leaves in contrast to only 23% in RH leaves. In addition, the Fₘ/Fₘ of RH leaves prior to the photoinhibitory treatment was slightly lower (0.74 ± 0.03) than that observed at time zero for RNH leaves (0.79 ± 0.01). These results are consistent with those recently published for attached RH and RNH leaves (25).

To test the hypothesis that the site for the development of resistance to low-temperature photoinhibition was PSII in rye thylakoids, we isolated thylakoids from RH and RNH leaves and exposed them to photoinhibitory conditions. Fₘ/Fₘ ratios of control samples taken from different experiments varied
between 0.65 and 0.74 for RNH thylakoids and from 0.61 to 0.64 for RH thylakoids prior to photoinhibitory treatment. Although the absolute values of $F_v/F_m$ observed for isolated thylakoids were about 15% lower than those observed in vivo, the lower values for RH thylakoids than for RNH thylakoids are consistent with that observed in vivo (Fig. 1) (25). The lower $F_v/F_m$ ratios in RH thylakoids prior to photoinhibitory treatment were essentially due to higher $F_o$ values, and their $F_v$ values were similar to those of RNH thylakoids (Fig. 2B and C). Regardless, the kinetics for the decrease in $F_v/F_m$ upon exposure to 2600 $\mu$mol m$^{-2}$ s$^{-1}$ at 20°C was similar for RH and RNH thylakoids during 1 h of this photoinhibitory treatment (Fig. 2A). After 60 min, $F_v$ could no longer be detected and, as a consequence, $F_v/F_m$ had decayed to zero. The observed decrease in $F_v/F_m$ was light dependent because, in the dark at 20°C, $F_v/F_m$ of RH and RNH thylakoids decreased by only 15% after 1 h (data not shown). A more detailed examination of the fluorescence parameters during the photoinhibitory treatment indicated that the decrease in $F_v/F_m$ was due to a decrease in $F_v$ over the 60 min treatment period coupled with a substantial increase in $F_o$ during the first 30 min (Fig. 2B and C).

For comparative purposes, the fluorescence data for RH and RNH thylakoids were normalized (Fig. 2D and E). The results showed that RH thylakoids exhibited a 25% increase, whereas RNH thylakoids exhibited a 40% increase in $F_o$ after 30 min of photoinhibitory treatment (Fig. 2E). However, the $F_v$ of RH thylakoids tended to be lower than that of RNH thylakoids during the photoinhibitory period (Fig. 2E). When the effects on $F_v$ and $F_o$ were combined to calculate $F_v/F_m$ (Fig. 2D), RH thylakoids tended to be more sensitive to the photoinhibitory treatment than RNH thylakoids. Similar trends were observed when lower PPFD (1000 $\mu$mol m$^{-2}$ s$^{-1}$) were used for the photoinhibitory treatment at 20°C or when RH and RNH thylakoids were isolated and resuspended in high osmoticum (0.8 m sorbitol) to stabilize the rye membranes (13) during the photoinhibitory treatment (data not shown).

In vivo exposure of attached rye leaves or rye leaf segments to moderate irradiance and low temperature markedly increased susceptibility to photoinhibition compared with the same light treatment at 20°C (25). In contrast to these in vivo observations, isolated RH and RNH thylakoids appeared to be less sensitive to photoinhibition at 5°C than at 20°C, as indicated by a slower rate of change in $F_v$, $F_o$, and $F_v/F_m$ during photoinhibition at 5°C (Fig. 3A and B). Similar observations have been reported previously (22, 29). The relative trends between RH and RNH thylakoids with respect to $F_v$, $F_o$, and $F_v/F_m$ observed during photoinhibition at 5°C were consistent with those observed at 20°C. Again the $F_v/F_m$ of RH thylakoids tended to be more sensitive to the photoinhibitory treatment than that of RNH thylakoids (Fig. 3A).

The photoinhibitory decrease in $F_v/F_m$ in RH and RNH thylakoids implies a decrease in the photosynthetic efficiency of PSII. This was corroborated by independent measurements of PSII electron transport under light-saturating (Fig. 4A) or light-limiting conditions (Fig. 4B). Both RH and RNH thylakoids exhibited similar kinetics for the photoinhibitory decrease in PSII activity, with RH thylakoids tending to be more sensitive to the photoinhibitory treatment than RNH thyla-
Figure 3. The effect of photoinhibitory treatment at 5°C on the fluorescence characteristics of RH and RNH thylakoids. All data have been normalized for convenience. Thylakoids were exposed to 2600 μmol m⁻² s⁻¹. Initial F₄/F₆ were 0.64 ± 0.01 (RH) and 0.74 ± (RNH). Most ± values were smaller than the symbol size.

Figure 4. Changes in the light-saturated (3000 μmol m⁻² s⁻¹) (A) and light-limited (250 μmol m⁻² s⁻¹) (B) rates of PSII electron transport in rye thylakoids as a function of exposure time to 2600 μmol m⁻² s⁻¹ at 20°C. Initial rates of PSII electron transport as measured at 25°C under light-saturating conditions were 473 ± 108 (RH) and 399 ± 53 (RNH) μmol DCPIP reduced mg Chl⁻¹ h⁻¹ and 89 ± 8 (RH) and 84 ± 11 (RNH) μmol DCPIP reduced mg Chl⁻¹ h⁻¹ under light-limiting conditions. Most ± values were smaller than the symbol size.

In addition, after 15 min at 2600 μmol m⁻² s⁻¹ at 20°C, the initial slopes of the light response curves for PSII activity for RH thylakoids exhibited a 2.5-fold decrease, whereas RNH thylakoids exhibited a 1.4-fold decrease in initial slope (Table I).

Concomitantly, light response curves for PSII electron transport were measured in RH and RNH thylakoids before and after photoinhibitory treatment at 2600 μmol m⁻² s⁻¹ and 20°C. The results in Table I indicate that both RH and RNH thylakoids exhibited a similar 2.7-fold decrease in the initial slopes for PSI electron transport as a consequence of the photoinhibitory treatment.

Table I. Initial Slopes of the Light Response Curves for PSII and PSI Activities of Control and Photoinhibited RH and RNH Thylakoids

<table>
<thead>
<tr>
<th>Sample</th>
<th>PSII²</th>
<th>PSI²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RH</td>
<td>RNH</td>
</tr>
<tr>
<td>Control</td>
<td>0.35 ± 0.03</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Treated</td>
<td>0.14 ± 0.01</td>
<td>0.26 ± 0.04</td>
</tr>
</tbody>
</table>

*μmol DCPIP reduced mg Chl⁻¹ h⁻¹/μmol photons m⁻² s⁻¹.  **μmol O₂ consumed mg Chl⁻¹ h⁻¹/μmol photons m⁻² s⁻¹.
observed in vivo. Thus, we examined RH and RNH thylakoids that had been isolated from rye leaves exposed to a photo-inhibitory treatment at 5°C and 1200 μmol m⁻² s⁻¹. The \( F_\text{m} / F_\text{m} \) of RNH and RH leaves was 0.77 ± 0.01 and 0.73 ± 0.02, respectively, prior to the photoinhibitory treatment. The \( F_\text{m} / F_\text{m} \) of isolated thylakoids from the control leaves was 0.73 ± 0.01 and 0.66 ± 0.01 for RNH and RH, respectively. As expected, after 5.5 h at 5°C and 1200 μmol m⁻² s⁻¹, the \( F_\text{m} / F_\text{m} \) of RH leaves was reduced by 61 ± 5% of the initial control values, whereas the \( F_\text{m} / F_\text{m} \) of RH leaves was reduced by only 37 ± 4%. In contrast, thylakoids isolated from these same leaves exhibited similar reductions in \( F_\text{m} / F_\text{m} \) of about 30% compared to control thylakoids. Thus, although RH and RNH leaves exhibited a differential sensitivity to photoinhibition, thylakoids isolated from the same leaves did not.

**DISCUSSION**

A combination of low temperature and moderate to high irradiance can induce photoinhibition in intact leaves as indicated by a decrease in \( F_\text{m} / F_\text{m} \) and in the quantum yield for \( O_2 \) evolution (11, 23, 24, 26, 32, 37). However, recently it has been reported that cold hardening of spinach (5, 30, 32) and winter rye (25) (Fig. 1) and winter wheat (Hurry, NPA Huner unpublished observation) results in the unique capacity to exhibit increased resistance to low temperature-induced photoinhibition. Thus, under conditions of low temperature and moderate to high light (400–1500 μmol m⁻² s⁻¹), non-hardened leaves exhibit a twofold greater suppression of \( F_\text{m} / F_\text{m} \) than cold-hardened leaves exposed to the same photo-inhibitory regimen. This differential change in \( F_\text{m} / F_\text{m} \) implicates PSII as the principal site of resistance. Hence, it seems reasonable to assume that the cold hardening-induced resistance to low temperature photoinhibition would be associated with thylakoid membranes. It has been suggested that the resistance induced by cold hardening is a function of reported changes in carotenoid composition of thylakoid membranes and the level of scavengers of active \( O_2 \) species in spinach chloroplasts (30). Furthermore, it has been suggested by Huner and co-workers (15) that the organizational changes observed in RH thylakoids may be related to an increased resistance to low temperature-induced photoinhibition. On the basis of in vitro Chl \( a \) fluorescence, PSII electron transport, atrazine binding, and PAS, the observed in vivo differential resistance to photoinhibition is not apparent at the level of isolated thylakoid membranes. Similar conclusions were reached whether isolated thylakoids were photoinhibited or whether thylakoids were isolated from photoinhibited leaves. Thus, we conclude that the mechanism of resistance to photoinhibition must require a higher level of cellular organization. Recently, we have shown that isolated, intact mesophyll cells from RH leaves are indeed more resistant to low temperature photoinhibition than intact mesophyll cells from RNH leaves based on fluorescence analyses (L. Lapointe, NPA Huner, manuscript in preparation). This indicates that increased resistance to photoinhibition probably involves complex regulation of PSII such that higher PSII efficiency is maintained in RH dark-adapted samples after exposure to a photoinhibitory event. This will be the subject of a forthcoming paper.

The in vitro results presented here indicate that thylakoids exposed to photoinhibitory conditions at either 5 or 20°C exhibited a significant increase in \( F_\text{r} \) (Figs. 2 and 3). Furthermore, RNH thylakoids exhibited a 40% increase in contrast to the 25% increase in RH thylakoids. An increase in \( F_\text{r} \) may reflect a decrease in the efficiency of energy transfer from LHCCI to PSII reaction centers as a result of physical dissociation of LHCCI from the PSII core (3). The differential effects of photoinhibition on \( F_\text{r} \) may reflect the organizational differences reported between RH and RNH LHCCI (14, 19). A closer association between LHCCI and PSII in RNH than RH thylakoids could explain the lower \( F_\text{r} \) and higher \( F_\text{m} / F_\text{m} \) in RNH than RH leaves and thylakoids. However, it is clear from the experiments presented here that these organizational differences in RH and RNH thylakoids cannot account for the differential resistance to photoinhibition observed in vivo.

It is interesting to note that photoinhibition appears to have a greater effect on the \( F_\text{m} / F_\text{m} \) of intact leaves than on the \( F_\text{m} / F_\text{m} \) of thylakoids isolated from the same leaves. This may be due to the fact that only the uppermost layer of mesophyll cells is detected by in vivo fluorescence, whereas thylakoids isolated from the same leaves reflect the total population of mesophyll cells within the leaf. Those thylakoids obtained from deeper within the leaf would be less photoinhibited than those closer to the exposed leaf surface.

Although PSI is stable to photoinhibition in vivo (17), the results in Table 1 indicate that the initial slopes for the light response curves for RH and RNH thylakoids decreased 2.7-fold after exposure to high light at 20°C for 30 min. Thus, in vitro the apparent quantum efficiency of both PSII and PSI are reduced by photoinhibition. The susceptibility of PSI to photoinhibition in vitro has been reported previously (3, 29). Clearly, photoinhibition in vitro is quite distinct from photoinhibition in vivo.

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