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Photoinhibition of Photosystem I in field-grown barley (*Hordeum vulgare* L.): Induction, recovery and acclimation

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Abstract

The effects of exposure of a field-grown winter cultivar of barley (*Hordeum vulgare* L.) to Photosystem I (PS I) photoinhibitory conditions in the form of bright day-light combined with chilling conditions were investigated. PS I photoinhibition was manifested by damage to the Fe-S centers of PS I and to the PS I-A/B polypeptides. Up to 20% of the PS I complexes were photoinactivated. Upon transfer to room temperature, the plants partially recovered from PS I photoinhibition, although damage was still detectable after one week. These results demonstrate that PS I photoinhibition is a physiologically relevant phenomenon in chilling-tolerant plants grown under field conditions. In order to study the induction of cyclic electron transport around PS I by PS I photoinhibitory conditions, antibodies raised against the NDH-I subunit of the NDH complex (a component of cyclic electron transport) were used to measure NDH levels in the exposed plants. A marked increase in the amount of NDH complex and a corresponding increase in NADPH dehydrogenase activity in the thylakoids were observed. The data indicate that the response to PS I-photoinhibitory conditions may involve regulated changes in cyclic electron transport around PS I.

Abbreviations: A_0 and A_1 – primary and secondary electron acceptors of Photosystem I; Chl – chlorophyll; F_A F_B and F_X – [4Fe-4S] iron–sulfur clusters of Photosystem I; NDH – NAD(P)H dehydrogenase; PS I – Photosystem I

Introduction

Photoinhibition is defined as the decrease in photosynthetic activity induced by light. When the harvested light energy exceeds the requirements to drive photosynthesis, and when the overall capacity of the protective mechanisms available are exceeded, photoinhibition may take place (Powles 1984; Barber and Andersson 1992; Aro et al. 1993; Foyer et al. 1994; Huner et al. 1998). Based on the observation that PS I is more stable than PS II during strong light treatments (Powles 1984) and that PS I photoinhibition has seldom been observed *in vivo* (Havaux and Eyletters 1991), the major site of photoinhibition has been assigned as PS II. However, in isolated thylakoid membranes, PS I has been shown to be as susceptible as PS II to photoinhibition (Satoh 1970a,b; Inoue et al. 1986, 1989; Sonoike 1995; Tjus 1995). Terashima et al. (1994) and Sonoike et al. (1995, 1997) showed that in chilling sensitive plants (cucumber) exposed to chilling temperature (≈4 °C) and moderate light (≈200 μmol photons m^{-2} s^{-1}) PS I is selectively photoinhibited. In accordance with the earlier data of Inoue et al. (1986), Sonoike et al. (1995) showed that this photoinhibition of PS I involved destruction of the iron–sulfur centers F_X, F_A and F_B, which are the likely sites of active oxygen formation. Furthermore, the PS I-B reaction center subunit of PS I was degraded by a combination of active oxygen species and a serine-type protease (Sonoike 1996a; Sonoike et al. 1997). Preferential photoinhibition of PS I has also been observed in potato which is less chilling sensitive than cucumber (Havaux and Davaud 1994). Recently, PS I photoinhibition was also observed in cold tolerant plants (Ivanov et al. 1998a; Tjus et al. 1998a, b, 1999). Chilling-resistant barley suffered from PS I photoinhibition after 4 h of low light treatment at low temperatures (Tjus et al. 1998a, 1999). The photoinhibition was revealed as damage to the iron–sulfur centers F_A, F_B, and F_X, and degradation of the reaction center polypeptides, PS I-A and PS I-B (Tjus et al. 1999). For recent reviews of PS I photoinhibition, see Sonoike (1996b, 1998a). Since PS I photoinhibition of chilling-resistant plants took place under conditions that are regularly experienced by plants in temperate climates, this inhibition could be important e.g. for crop growth range and productivity.

Cyclic electron transport is a process which has been suggested to play an important role in protecting against photooxidative stress (Heber and Walker 1992; Fork and Herbert 1993). Cyclic electron transport is the light-mediated transfer of electrons from the plastoquinone pool, via PS I and ferredoxin, back to the plastoquinone pool (Bendall and Manasse 1995). Cyclic electron transport may reduce the photoinhibitory effect of pseudocyclic electron transport (i.e. the photoreduction of oxygen) via the Mehler reaction, by re-routing linear electron transport. Furthermore, acidification of the thylakoid lumen by proton translocation during cyclic electron transport can increase non-photochemical quenching by different mechanisms and thus prevent over-reduction of the electron carriers and subsequent photoinactivation of the electron transport chain (Demmig-Adams and Adams 1992; Heber et al. 1992; Katona et al. 1992; Topf et al. 1992; Van Wijk and Van Hasselt 1993; Van Voorthuysen et al. 1996). In agreement with this proposed role of cyclic electron transport, the increase of cyclic electron transport following exposure of cucumber to PS I-photoinhibitory conditions, i.e. chilling and low-light, has been reported (Herbert et al. 1997; Sonoike 1998b, 1999). A recent investigation has shown that PS I in tobacco was very sensitive to photoinhibition in the presence of antimycin A, which is an inhibitor of cyclic electron transport (Chow and Hope 1998). Thus, cyclic electron transport may be a particularly important factor in preventing the specific photoinactivation of PS I.

The specific pathways for cyclic electron transport are poorly understood but apparently several pathways operate in parallel (Bendall and Manasse 1995; Scheller 1996). The thylakoidal NAD(P)H dehydrogenase (NDH) complex has been suggested to be involved in cyclic electron transport in both plants (Sazanov et al. 1998; Teicher and Scheller 1998) and cyanobacteria (Mi et al. 1995). NDH proteins have been detected as thylakoid membrane components located in the non-appressed stromal lamellae (Nixon et al. 1989; Berger et al. 1993; Steffánsson 1996) and in bundle sheath chloroplasts of C4 plants (Kubicki et al. 1996), the site of cyclic electron transport in these species. These findings are in agreement with a function of the NDH complex in cyclic electron transport. The thylakoidal NDH complex has been implicated in regulating responses to salt stress in cyanobacteria (Hibino et al. 1996), senescence (Cuello et al. 1995) and photooxidative stress (Herbert et al. 1995; Martin et al. 1996; Catalá et al. 1997). Martin et al.(1996) and Catalá et al. (1997) have presented evidence for upregulation of NDH-A and NDH-F, respectively, in the response of barley to photooxidative stress, while Herbert et al. (1995) have shown that cyanobacteria grown under high light intensities have increased rates of NDH-mediated cyclic electron transport, and that these increased rates are a response to chronic photoinhibition. In the dark, the NDH complex may also facilitate the generation of ATP by chlororespiration (Bennoun et al. 1982; Garab et al. 1989; Bennoun 1994) using a stromal reductant such as NADH (Corneille et al. 1998; Sazanov et al. 1998) which in turn could be produced by glycolysis. This would give the plant the advantage of creating a transthylakoid pH gradient independently of light-mediated linear electron transport, permitting chloroplast metabolism in the dark as well as redox regulation under stress conditions.

In the present study, we demonstrate that PS I photoinhibition is significant under field conditions and correlate these findings to the role of cyclic electron transport around PS I, and document a substantial upregulation of the NDH complex in response to photoinhibitory conditions.

Materials and methods

Plant material and illumination conditions

Winter barley (*Hordeum vulgare* L. cv. Tiffany) was germinated indoors in a soil/sphagnum mixture (50/50 v/v). Fluorescent tubes provided a PPFD of 80 μmol m^{-2} s^{-1}. For indoor studies, barley was grown in the germination chamber for additional 7–14 d at room temperature (-20 °C). Indoor growth took place using a 12-h photoperiod. For outdoor studies, plants were germinated indoors as described, and were transferred to field plots at 7 d post germination. Outdoor plants grew in the light, or were protected from sunlight by metal foil-covered pots for 7–14 d before sampling. The covered plants did not exhibit loss of chlorophyll from the already green parts during the experiment. One plot of outdoor plants grew in the light for 7 d and was subsequently transferred to indoor growth conditions for a further 7 d prior to sampling. Outdoor planting was carried out in April, i.e. early spring. Naturally acclimatized plant material was obtained from plants planted outside in October and sampled at the same time as the other plants in the study. Local temperatures and irradiances were measured prior to and during the sampling period and plots were covered in the event of frost. Temperature fluctuations measured directly at plant level were in the range -0.2 °C to +16 °C, while irradiances varied between 0 μmol m^{-2} s^{-1} and 630 μmol m^{-2} s^{-1}. Peak irradiances during overcast days where as much as 50% lower than during clear days. Approximately 2 h after sunrise, the temperature was generally 4–6 °C, while the irradiation was 100–200 μmol $m^{-2}s^{-1}$. These conditions approximate the optimal conditions for PS I photoinhibition in cucumber reported by Terashima et al. (1994) and Sonoike et al. (1995, 1997). Leaves were harvested 2 h after sunrise; only the upper 9 cm of the leaves, i.e. green and mature parts of corresponding age, were harvested. Thylakoid membranes were isolated (Teicher and Scheller 1998) immediately after harvest of the leaves, frozen in liquid nitrogen and stored at -80 °C until assayed. Chlorophyll was determined according to Arnon (1949).

Electrophoresis and immunoblotting

Thylakoid proteins were separated by SDS–PAGE using 8–25% linear gradient gels (Fling and Gregerson 1986). Thylakoids were completely solubilized at 70 °C for 20 min with sample buffer containing 1% SDS and 20 mM DTT prior to electrophoresis. Immunoblotting was carried out using nitrocellulose membranes incubated with polyclonal rabbit antibodies against barley PS I-A/B (Andersen et al. 1992) or against tobacco NDH-I fusion proteins (Burrows et al. 1998). A chemiluminescence detection system (SuperSignal^{TM}, Pierce, Rockford, Illinois) was used to visualize the antibody reactions.

PS I activity measurements

The degree of PS I intactness was determined from flash-induced P700 absorption changes at 834 nm (Naver et al. 1996; Tjus et al. 1998a) and was calculated using an extinction coefficient of 5 $mM^{-1}cm^{-1}$. Saturating actinic light (532 nm, 6 ns FWHM, 1 Hz) was provided by a Nd:YAG laser (Quanta Ray model GCR-100, Spectra Physics Lasers Inc., Mountain View, California). Thylakoid membranes (8 μg of Chl) were dissolved in 300 μl reaction mixture containing 20 mM Tricine (pH 7.5), 0.065% (w/v) n-decyl-β-D-maltopyranoside, 670 μM sodium ascorbate and 20 μM 2,6-dichlorophenolindophenol. Typically, 32 absorbance transients were collected and averaged for each decay curve. The recorded signals were resolved into exponential decay components by Levenberg-Marquardt non-linear regression (Press et al. 1989). Analysis of the averaged absorbance transients was carried out according to Tjus et al. (1998a). Excessive solubilization may lead to absorbance transients from triplet chlorophyll. To ensure that triplet chlorophyll did not contribute to the absorbance transients, the light saturation response of the different absorbance decay components was investigated as previously described (Tjus et al. 1998a). As in the previous study, all decay components had the same light saturation response indicating that all were derived from P700 with no contribution from triplet chlorophyll.

NDH activity measurements

Quantification of light-dependent NAD(P)H oxidation at 340 nm was carried out essentially according to Teicher and Scheller (1998). The reaction mixture (0.5 mL) contained 24 mM Tris-HCl (pH 8.6), 48 mM NaCl, 200 μM NADPH, 6 mM NH_4Cl, 100 μM methyl viologen, 10 μM DCMU, and thylakoids corresponding to 4 μg Chl. The oxidation of NADPH at 25 °C was followed in an Aminco DW-2000 spectrophotometer with a thermostated cuvette holder and was calculated using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$. Actinic light was provided by a KL1500 lamp (Schott) with a RG650 filter (Schott) providing about 1000 μmol photons m^{-2} s^{-1}.

Fluorescence measurements

Determination of fluorescence parameters (F_o and F_m) was carried out using a PAM 101–103 fluorometer (Walz, Effeltrich, Germany). Thylakoid membranes

Table 1. Characterization of the thylakoid membranes used in this study. Values are shown as mean ± SE (n = 3), except for ΦPS II (quantum yield of PS II), which in all cases had SE < 0.01. Abbreviations used include: C20°, indoor control; D5°, outdoor dark; L5°, outdoor light; Accl, acclimatized; L5°–20°, out- to in-door transfer. For further details, refer to 'Materials and methods'

		Chl *a/b*	ΦPS II
7-d exposure	C20°	2.67 ± 0.06	0.69
	D5°	2.82 ± 0.04	0.68
	L5°	2.95 ± 0.06	0.60
	Accl	2.87 ± 0.03	0.53
14-d exposure	C20°	2.49 ± 0.14	0.68
	D5°	2.66 ± 0.14	0.75
	L5°	2.82 ± 0.10	0.57
	L5°−20°	2.95 ± 0.02	0.65

(50 μg Chl ml^{-1}) were suspended in a buffer containing 20 mM Tricine (pH 7.5), 10 mM NaCl, 5 mM mgCl$_2$ and 0.4 M sucrose. F$_o$ was determined with the measuring light below 0.1 μmol m^{-2} s^{-1}. Saturating pulses of white light (800 ms, 6000 μmol m^{-2} s^{-1} were applied from a Schott / Walz KL-1500 FL103F lamp to determine F$_m$. The variable fluorescence parameter F$_v$ was calculated as F$_v$ = (F$_m$ − F$_o$). The maximal quantum yield of PS II (ϕPS II) was calculated as F$_v$ / F$_m$.

Results

Exposure to chilling conditions in the light causes photoinhibition of both PS II and PS I

Photoinhibition of PS II was determined from the maximal quantum yield of PS II (Table 1). Indoor grown plants (C20°), as well as outdoor grown plants protected from light (D5°) contained relatively intact PS II complexes (F$_v$/F$_m$ $\approx$ 0.7). Outdoor grown plants exposed to light (L5°) had approximately 18% lower F$_v$/F$_m$ ratios than outdoor grown plants protected from light. The decrease in F$_v$/F$_m$ was more pronounced after 2 weeks of exposure, compared to 1 week of exposure.

P700 absorption decay kinetics were measured to monitor the status of PS I (Figures 1 and 2). Within the time resolution of the experimental setup, it was possible to identify intact PS I complexes (> 30 ms backreaction from [F$_A$/F$_B$]$^-$ to P700), PS I with damaged F$_A$/F$_B$ (1 ms backreaction from F$_X$$^-$) and PS I with damage to F$_X$ (approximately 5 μs backreaction from A$_1$$^-$). Further damage to the PS I electron transfer chain is manifested as a loss in total amplitude. It should be noted that accurate resolution of the total amplitude (i.e. the amount of photooxidizable P700) was not possible due to the fact that the time resolution of the experimental system does not permit measurement of the extremely rapid backreaction from A$_0$$^-$ to P700$^+$ which takes place in PS I when also A$_1$ has been damaged. For a detailed review on backreaction rates in PS I see Brettel (1998). To deconvolute P700 decay kinetics into fast (μs) and slow (ms) components, time windows of 500 μs and 20 ms were analyzed. Figure 1 shows typical examples of the primary data obtained. The sample from indoor control plants (Figures 1A, C) showed only the slow decay of about 30 ms indicating intact electron acceptors. In contrast, in a sample from plants exposed to chilling and light for 7 d, about 10% of the absorbance change decayed with the 5 μs time constant indicating a loss of functional iron-sulfur clusters (Figure 1D). Analysis of the photoinhibited sample in the 20 ms time window showed that the 1 ms decay was absent (Figure 1B). Thus, in this particular sample no fraction containing functional F$_X$ but damaged F$_A$/F$_B$ was present. Data obtained in this way were used to compile Figure 2 which reveals that damage to the electron acceptors was essentially absent in indoor grown plants (C20°), as well as in outdoor grown plants protected from the light (D5°). A fast μs decay was observed in outdoor grown plants exposed to light (L5°), acclimatized plants (Accl) and in plants transferred from outdoor growth to indoor growth conditions (L5–20°). An additional 1 ms component was only identified in outdoor grown plants exposed to the light for 14 days (L5°; Figure 2). The damaged PS I that was detected constituted 15% and 20% of the intact PS I after 1 and 2 weeks exposure, respectively. Transfer of the barley plants from outdoor to indoor growth conditions diminished PS I damage to 5% of the intact PS I. The response of barley PS I to growth conditions was also studied by immunoblot analysis using antibodies raised against barley PS I-A/B (Figures 3A, B). Outdoor grown plants exposed to light (L5°), as well as acclimatized plants (Accl) showed a decrease in the amount of intact PS I-A/B protein, as opposed to indoor grown controls (C20°), outdoor grown light-protected plants (D5°) and plants transferred from outdoor growth to indoor growth con-

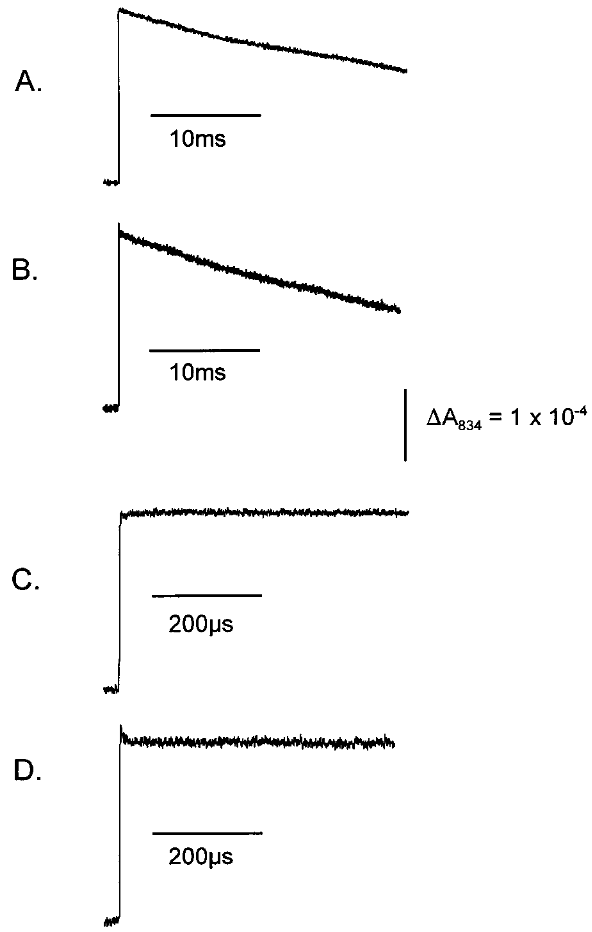

Figure 1. Flash-induced absorption changes (ΔA_{834}) of P700 in isolated barley thylakoids. Transients representing μs and ms time windows of absorption changes are presented. (A) and (C) Thylakoids from indoor control plants. (B) and (D) thylakoids from photoinhibited plants exposed to light for 7 d. For further details refer to 'Materials and methods'.

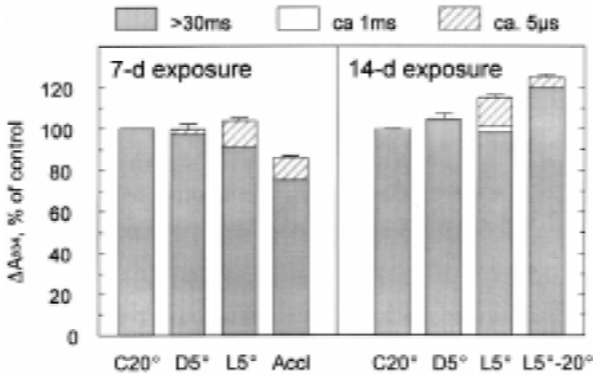

Figure 2. Kinetics of flash-induced absorption changes (ΔA_{834}) of P700 in isolated barley thylakoids. The slow time component (>30 ms) represents the back-reduction from the Fe-S centers F_A/F_B of the intact PS I complex. The 1 ms time component represents inactivation of the Fe-S centers F_A/F_B. The 5 μs time component reflects inactivation of the Fe-S centers F_A/F_B and F_X. Error bars (+SE) indicate variances for the total absorption amplitudes (n = 3–5). Symbols: C20° – indoor control; D5° – outdoor dark; L5° – outdoor light; Accl, acclimatized; L5°–20°, out–in-door transfer.

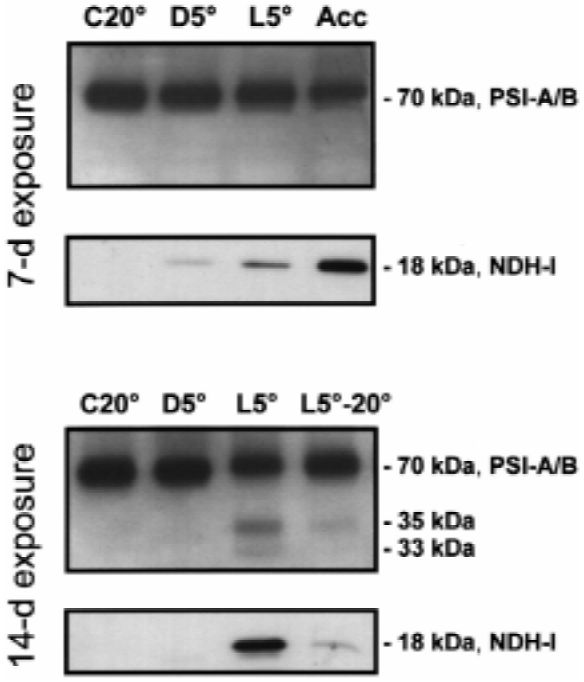

Figure 3. Immunoblot analysis of barley thylakoids using specific antibodies against PS I A/B (top) and NDH-I (bottom). Thylakoids corresponding to 3 μg Chl were applied to each lane. Symbols: C20° – indoor control; D5° – outdoor dark; L5° – outdoor light; Accl – acclimatized; L5°–20°, out–in-door transfer.

ditions (L5–20°). In addition to the observed decrease in the amount of PS I-A/B protein, PS I-A/B antibodies recognized two additional bands (35 kDa and 33 kDa) in plants exposed to outdoor growth conditions for 14 days (Figure 3B), and to a lesser extent in plants transferred from outdoor growth to indoor growth conditions (L5–20°). The data reported here derive from experiments carried out in 1998. Similar degrees of photoinhibition of PS I were obtained in field experiments carried out in 1995–1997.

Chl a/b ratios change in response to outdoor growth conditions

The Chl *a/b* ratio in the barley thylakoids was determined to detect possible changes in chlorophyll composition of the light harvesting complexes in response to growth conditions (Table 1). A slight increase in Chl *a/b* ratio was observed in plants exposed to outdoor conditions, as opposed to indoor-grown controls

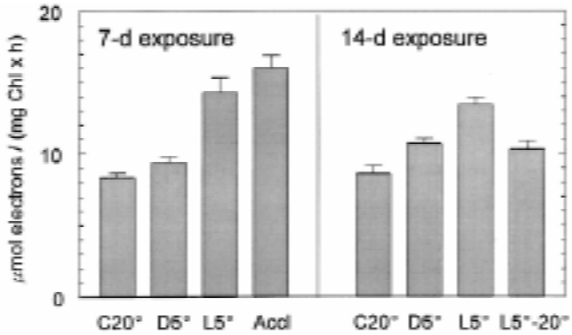

Figure 4. Rates of light-dependant NADPH oxidation (DA$_{834}$) in barley thylakoids. The thylakoids were incubated for 30 min in the dark in the presence of the assay reagents, after which time the NDH activity (± SE, $n = 3$) was assayed. Symbols: C20° – indoor control; D5° – outdoor dark; L5° – outdoor light; Accl – acclimatized; L5°–20° – out–in-door transfer.

(C20°). The increase indicates that the PS I/PS II ratio was increased, or that the antenna size was decreased in outdoor grown plants. In agreement with the observed change in Chl *a/b* ratios, it is apparent from the P700 absorption amplitudes (Figure 2) that there has been a slight decrease in Chl/P700 ratio in the outdoor grown plants. The acclimatized plants are an exception since the P700 absorption was low while the Chl *a/b* ratio did not differ from the other outdoor grown plants. The loss of P700 absorption amplitude in the acclimatized plants may indicate damage to PS I acceptors functioning earlier than the iron–sulfur clusters. If the absorbance change from intact PS I in the acclimatized plants is compared to the total absorption in the other outdoor grown plants it can be estimated that about 30% of PS I in the acclimatized plants was inactivated.

Photooxidative stress induces NDH

The effect of altered growth condition on the thylakoidal NAD(P)H dehydrogenase was determined by immunoblot analysis, using a specific antibody against the NDH-I subunit (Figures 3A, B). NDH levels were substantially increased in plants exposed to outdoor growth conditions, particularly after 14 d of exposure and in acclimatized plants. NDH levels in outdoor grown plants protected from light (D5°) were not significantly higher than those observed in control plants. NDH levels in plants transferred from outdoor to indoor growth conditions (L5–20 °) showed a reversion to the low levels of NDH characteristic of indoor grown plants. NDH activity was determined as the light mediated oxidation of NADPH (Figure 4). In agreement with the immunoblot studies, NDH activity was increased in plants exposed to outdoor growth conditions for 7 d and 14 d (Figure 4, L5°) compared to indoor-grown controls (C20°). However, the differences in activities were smaller than the differences in NDH-I content. NDH activity was also increased in acclimatized plants (Accl) compared to indoor-grown control plants.

Discussion

Exposure of barley plants to outdoor growing conditions (chilling temperatures and relatively low light) lead to PS I photodamage, as revealed both by kinetics of the flash-induced P700 absorbance changes and by immunoblotting (Figures 1–3). The level of PS II photoinhibition as determined by the decrease in ΦPS II (Table 1) follows the PS I photoinhibition. This is in agreement with our previous studies with barley, where conditions that specifically inhibit PS I were not found (Tjus et al. 1998a). In chilling-sensitive species at chilling temperatures, the situation may be different. Thus, under low-light illumination of cucumber plants (Terashima et al. 1994,1998) as well as high-light illumination of potato plants (Havaux and Davaud 1994) it has been demonstrated that PS I is more susceptible to photoinhibition than PS II.

The clearest sign of a photodamage to PS I-A/B is observed in Figure 3B (L5°). The appearance of two polypeptides of approximately 33 kDa and 35 kDa shows that PS I-A/B is degraded following outdoor growth at chilling temperatures for 14 days. Tjus et al. (1999) demonstrated that both PS I-A and PS I-B were damaged upon PS I photoinhibition, and Sonoike (1996a) and Sonoike et al. (1997) showed that PS I-B was destroyed by a combination of active oxygen species and a serine-type protease. It is apparent that the proteolytic degradation of damaged PS I-A/B is not completed at low temperature with a resulting transient accumulation of the 33 and 35 kDa fragments. Upon transfer to higher temperatures the accumulated fragments are further degraded (Figure 3B, L5°–20°)

Flash-induced P700 absorption changes at 834 nm (Figure 2) correlate with PS I damage detected by the apparent decrease in PS I-A/B protein levels (Figure 3). PS I damage as envisaged by these flash-induced P700 absorption changes at 834 nm revealed that the maximum degree of inhibition is about 15–20%. The low total absorption amplitudes of P700 in the acclimatized plants suggest that damage to PS I acceptors preceding the iron–sulfur clusters has taken place. In these plants, the degree of inhibition is approximately 30%. Apparently, the acclimatized plants accumulated damaged PS I, which was not turned over under the outdoor conditions.

In addition to the occurrence of PS I photodamage following exposure of barley plants to outdoor growing conditions at chilling temperatures, the quantity as well as the activity of NAD(P)H dehydrogenase (Figures 3 and 4) increased. The increase in the levels of NDH-I protein was clearly light-stimulated, and was higher in plants exposed to outdoor growth conditions for 2 weeks (Figure 3B, L5°) than for 1 week (Figure 3A, L5°). Acclimatized plants showed similar high levels of NDH-I protein. NDH activity (Figure 4) could be seen to increase with levels of NDH-I observed in Figure 3. Thus, photooxidative stress induces the NDH complex. However, the degree of increase in NDH activity does not correspond to the observed degree of increase in NDH-I protein. A possible explanation could be that the amount of the NDH-I protein detected does not necessarily represent active NDH. The NADPH oxidizing activity of plants exposed to outdoor growth conditions for 2 weeks (Figure 4B, L5°) was lower than expected when compared to plants exposed to outdoor growth conditions for 1 week. A possible explanation could be that the NDH complex, and not just PS II and PS I, is susceptible to damage by active oxygen species. However, further experiments will be required to determine if this is the case. The upregulation of the NDH complex under PS I photoinhibitory conditions is in agreement with a role of this complex in cyclic electron transport and protection against photoinhibition. While the data presented here as well as several other investigations (Sonoike 1998b, 1999; Chow and Hope 1998) suggest a role of cyclic electron transport in protecting against photoinhibition of PS I, it is not possible to predict in quantitative terms the significance of this protection. Future investigations with plants modified in their ability to carry out cyclic electron transport will be required.

The documented occurrence of PS I photoinhibition under field conditions should be considered separately from photoinhibition induced only under extreme experimental conditions. The reported occurrence of PS I and PS II photoinhibition in winter cereals grown under temperate climatic conditions suggests that early-season biomass yields may be reduced. However, if biomass yield and thereby crop productivity are to be significantly decreased, carbon uptake must be depressed over a relatively long period. Photodamage to PS I or PS II would have the largest effect on photosynthetic carbon uptake under light-limiting conditions (below about 200 μmol m^{-2} s^{-1}). Such conditions prevail during at least 25% of the daylight period in the present study. The present study documents that recovery or turnover of damaged PS I is a slow process. Thus, photoinhibited plants allowed to recover for 7 d under optimal conditions still contained significant amounts of damaged PS I (Figure 2, L5°–20°). Preliminary studies indicate that no diurnal repair of PS I photoinhibition was detectable in the field under chilling conditions (H.B. Teicher and H.V. Scheller, unpublished data). Because recovery of PS I activity is slow, photosynthetic carbon uptake will indeed be limited. For example, PS I photoinhibition that takes place in the morning will not only limit carbon assimilation during the last hours of daylight on the same day, but will also affect carbon assimilation the following days. This situation is different from PS II photoinhibition, where recovery is completed in a matter of a few hours, if the temperature does not remain low. Long et al. (1994) have developed a model to predict the significance of PS II photoinhibition in nature. The model simulates a decline in carbon uptake by mid-afternoon, and a recovery to 90% of the optimal carbon uptake by dusk. The model predicted a 9% loss of carbon uptake, mainly in the late afternoon. However, the data presented in the present paper regarding the slow recovery of PS I from photoinhibition suggests that a model based on transient photoinhibition of PS II will only predict a part of the decrease in carbon uptake. It seems likely that PS I photoinhibition has a far greater significance for plant productivity in temperate climates than previously realized. Transfer of plants from outdoor, chilling conditions to indoor conditions indicates that photoinhibition in the field is reversed upon the onset of warmer temperatures (Table 1, Figures 2 and 3). However, early-season growth disadvantages could affect growth and development during the rest of the season. More detailed studies of the recovery from PS I photoinhibition, combined with a more accurate knowledge of the effect of PS I photoinhibition on biomass yields, would permit the generation of a model for the impact of photoinhibition on crop production and in nature.

In conclusion, PS I photoinhibition is predicted to have serious implications on productivity in field-grown crops under temperate growth conditions. In addition, regulated changes in cyclic electron transport around PS I appear to play a vital role in the alleviation of photoinhibition in field-grown crops under these conditions. While *in vitro* studies of cyclic electron transport and PS I photoinhibition are excellent tools for the elucidation of the mechanisms of these processes, the physiological role of cyclic electron transport in regulatory processes (including adaptation to environmental stresses) are best studied *in vivo*, in intact organisms and under natural and physiologically relevant conditions.

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