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Photoprotection of residual functional photosystem II units that survive illumination in the absence of repair, and their critical role in subsequent recovery

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Photosystem II (PSII) complexes, which split water into oxygen, protons and electrons in photosynthesis, require light but are also inactivated by it. Recovery of PSII from photoinactivation requires de novo protein synthesis. PSII in capsicum leaf segments were photoinactivated in the absence of chloroplast-encoded protein synthesis. At large photon exposures and despite the absence of repair, a residual fraction of PSII remained functional, being ca 0.08–0.2 depending on the ease of gas exchange in the tissue. This study revealed that the residual functional PSII was photoprotected by both (1) reaction-center quenching of excitation energy by photoinactivated PSII even when little or no PSII activity was permitted, and (2) antenna quenching, which was dependent on a trans-thylakoid pH gradient sustained mainly by linear electron transport and facilitated by the residual functional PSII complexes themselves. Significantly, little or no contribution to photoprotection of PSII was observed from cyclic electron flow around PSI. Further, the small residual functional PSII population was critical for recovery of the photoinactivated PSII complexes. Thus, photoinactivated and residual functional PSII complexes in leaves play a mutually beneficial role in each other's ultimate survival.

Introduction

Photosystem II (PSII) performs the unique function of light-induced water splitting to liberate electrons and protons for photosynthesis while releasing oxygen that is essential for aerobic organisms. Paradoxically, PSII is inactivated by light, being ''intrinsically suicidal'' (van Gorkom and Schelvis 1993). Photoinhibition is a general term that describes the light-induced loss of quantum efficiency of photosynthesis, because of photoinactivation of PSII and/or an enhanced dissipation of excitation energy as heat to prevent photoinactivation, i.e. photoprotection (Osmond et al. 1998). The quantum yield of photoinactivation of PSII is low, ca 0.1 μmol PSII mol^{-1} photons at the start of photoinactivation. However, the large number of photons incident over a sunny day (ca 30 mol m^{-2} in leaves) compared with the small number

Abbreviations – ATP, adenosine triphosphate; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; ΔpH, trans-thylakoid pH gradient; ETR, rate of electron transport through PSII; F_m, maximum Chl fluorescence yield of a dark-adapted leaf; F'_m, maximum Chl fluorescence yield of a light-adapted leaf; F_s, steady-state Chl fluorescence yield of a light-adapted leaf; F_o, minimum Chl fluorescence yield of a dark-adapted leaf; LHCII, light-harvesting Chl *a*/*b*–protein complexes in PSII; MV, methyl viologen; P700, special Chl pair in the PSI reaction center; $P700^+$, oxidized P700; PS, photosystem; SE, standard error of the mean.

of PSII complexes (ca 1 μmol PSII m^{-2}) means that most of the PSII complexes undergo photoinactivation during a sunny day. To minimize light-induced damage to PSII, plants have evolved numerous photoprotective molecular strategies (Demmig-Adams and Adams 1992, Horton et al. 1996, Niyogi 2000, Öquist and Huner 2003), as well as an elaborate repair mechanism for recovery from photoinactivation in weaker light (Aro et al. 2005, Mattoo and Edelman 1987, Mattoo et al. 1984).

Although photoinactivation of PSII is almost inevitable, it is a puzzle that a small residual population of functional PSII remains even when leaves are given prolonged illumination in the absence of repair (Lee et al. 2001, Norén et al. 1999, Park et al. 1997). How are the residual functional PSII complexes photoprotected? What function(s) do they serve? It has been proposed that one mechanism of photoprotection of the residual functional PSII complexes is reaction-center quenching, whereby photoinactivated PSII complexes act as efficient energy sinks, ameliorating photooxidative damage in and around themselves (Krause 1988) and dissipating excitation energy from functional neighbors connected via a common light-harvesting antenna (Ivanov et al. 2003, Matsubara and Chow 2004, Öquist et al. 1992). Consistent with this hypothesis, two populations of PSII have been observed *after* photoinhibition in vivo, representing strongly and weakly quenching PSII; these two populations of photoinactivated PSII were observed under conditions in which there was little or no linear electron flow through both PS (Matsubara and Chow 2004). Crucial questions remain, however. First, did PSI cyclic electron transport *during* the Chl *a* fluorescence lifetime measurement cause the energy dissipation in either strongly or weakly quenching PSII complexes via a trans-thylakoid pH gradient (ΔpH)? Second, in strong light and the presence of residual PSII activity *during* photoinhibition were the residual functional PSII complexes photoprotected by both antenna quenching and reaction-center quenching of excitation energy? Third, if the residual fraction of functional PSII complexes, small though it may be, survives prolonged illumination in the absence of repair, does it serve any purpose useful to the leaf? Our present investigation addressed these critical questions.

Materials and methods

Growth of plants

Capsicum annuum L. cv. Newtown No. 3 plants were grown at 24/21°C (day/night) with a 12-h photoperiod (150 μmol photons m^{-2} s^{-1}). The potting mixture was supplemented by a slow-release fertilizer, with the exception that the experiments in Fig. 5 were conducted with plants grown in Hoagland's solution.

Uptake of lincomycin

Detached leaves were allowed to take up lincomycin (3 m*M*) through the cut petiole for 3 h in darkness. Concentrations taken into the leaf tissue were ca 2 m*M*. Cut leaf segments were then floated on a lincomycin solution (1 m*M*) during photoinhibition.

Photoinhibition treatment

Leaf segments floating with the abaxial (lower) side in contact with 1 m*M* lincomycin (25°C) were illuminated on the adaxial side at 750–1300 μmol photons m^{-2} s^{-1}. Occasionally, as specified, the adaxial surface was in contact with lincomycin and illuminated from below, whereas the abaxial surface was exposed to air. Sometimes, to avoid the use of lincomycin, leaf segments were photoinhibited at ca 7°C to hasten the photoinactivation of PSII.

PSII functionality

In most cases, the functional PSII content was determined by flash-induced oxygen evolution, using repetitive single turnover, saturating xenon flashes and assuming that each functional PSII evolves one O_2 molecule after four flashes (Chow et al. 1989). A small heating artifact was corrected for. The O_2 yield/flash/Chl of photoinhibited samples was normalized to the value of the control to obtain the functional fraction of PSII.

In the experiments of Fig. 5, leaf pieces were dark adapted for 30 min prior to determination of the minimal (F_o) and maximal (F_m) fluorescence yield, corresponding to open and closed PSII reaction centers, respectively. F_o and F_m were measured with a Plant Efficiency Analyzer (Hansatech, King's Lynn, UK). All fluorescence yields were normalized to the mean F_o value of control leaf pieces. The relative content of functional PSII was calculated as $1/F_o - 1/F_m$, which is directly proportional to the oxygen yield per single turnover saturating flash in *C. annuum* (Lee et al. 2001).

Measurement of redox kinetics of P700

Redox changes of special Chl pair in the PSI reaction center (P700) were observed using a dual wavelength (810/870 nm) unit (ED-P700DW) attached to a phase amplitude modulation fluorometer (Walz, Effeltrich, Germany) and used in the reflectance mode (Chow and Hope 2004). To obtain redox changes because of a flash superimposed on

continuous far-red light, a steady state was sought by illumination with far-red light (12 μmol photons m^{-2} s^{-1}, peak wavelength 723 nm, 102-FR, Walz, Effeltrich, Germany) for ≥10 s. Then a single-turnover xenon flash (XST 103 xenon flash, Walz, Effeltrich, Germany) was applied to the adaxial side of the leaf disk. Flashes were given at 0.1 Hz, and eight consecutive signals were averaged (time constant = 95 μs). The maximum signal immediately after the flash was taken to represent the total amount of photooxidizable P700, and used to normalize the signals obtained in the same geometry with red or white actinic light (see below).

The redox kinetics of P700 before, during and after illumination with red light were measured under conditions that permitted little or no PSII activity, i.e. 120 μmol photons m^{-2} s^{-1}, −2°C and the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU). A photoinhibited leaf segment was vacuum infiltrated with DCMU (30 μ*M*) and kept in a cuvette in an aluminum block at −2°C. A window in the aluminum block allowed monitoring of redox changes of P700 in the reflectance mode. Red light from a light-emitting diode was turned on for 10.5 s and then off, while data acquisition (time constant = 2.3 ms) continued into the dark period. The red light illumination was repeated at 0.012 Hz, and six consecutive signals were averaged.

The redox kinetics of P700 before, during and after illumination with white light were measured under conditions similar to those used to photoinhibit leaf segments, i.e. 900 μmol photons m^{-2} s^{-1}, 25°C and the presence of lincomycin (but absence of DCMU). A photoinhibited leaf segment after the photoinhibition treatment was vacuum infiltrated with (1) water, (2) 300 μ*M* methyl viologen (MV), (3) 100 μ*M* DCMU or (4) 100 μ*M* DCMU + 300 μ*M* MV. Each leaf segment was dried in air in darkness for up to 30 min to remove excess intercellular water, and then preilluminated with white light (900 μmol photons m^{-2} s^{-1}) for 5 min. White light was turned on for 2 s and then off, while data acquisition (time constant = 95 μs) continued into the dark period. Illumination with white light was repeated at 0.083 Hz, and 16 consecutive signals were averaged.

The rate of electron transport through PSII measured by Chl *a* fluorescence

A control leaf segment was placed under the light guide of a phase amplitude modulation fluorometer, with a separation of about 5 mm to allow free air movement. Actinic light of varied irradiance was used to determine the relative Chl *a* fluorescence yield at steady state (F_s) and the maximum relative Chl *a* fluorescence yield in the light-acclimated state (F'_m). The average quantum yield of PSII photochemistry (Genty et al. 1989) ($1 - F_s/F'_m$) was used to calculate the rate of electron transport through PSII (ETR) as $(1 - F_s/F'_m) \times I \times 0.85 \times 0.5$ where I is the irradiance, 0.85 the assumed absorptance and 0.5 the assumed fraction of absorbed light partitioned to PSII.

ETR was determined by illuminating the adaxial leaf surface of a control leaf segment floating on water, with the abaxial surface either (1) exposed to air or (2) in contact with water. In case (1), the actinic light passed through a clear-perspex water bath from below to reach the adaxial surface.

Recovery from photoinactivation

To study recovery of PSII after photoinactivation, lincomycin was omitted during the light treatment (1000 photons m^{-2} s^{-1}), but small leaf segments were illuminated at ca 7°C to slow down repair and hasten net photoinactivation. After photoinactivation of a large fraction of PSII, leaf disks were floated with the abaxial surface in contact with a solution of 50 μ*M* DCMU (or water) in the dark for 30 min, with gentle stirring. Then they were allowed to recover in low light (20 μmol photons m^{-2} s^{-1}) at 25°C, while still floating on a solution of 50 μ*M* DCMU (or water). The fraction of functional PSII was determined from the Chl *a* fluorescence parameter $1/F_o - 1/F_m$ as described above.

Results

Residual functional PSII complexes during prolonged light stress

PSII functionality was monitored in leaf segments after photoinactivation in the presence of lincomycin, an inhibitor of repair that depends on chloroplast-encoded protein synthesis. The number of functional PSII complexes in non-photoinactivated leaf segments was 2.45 ± 0.05 mmol (mol Chl)$^{-1}$ [±standard error of the mean (SE), n = 7 leaf segments], a value taken to be the initial fraction 1.00 of functional PSII. Because photoinactivation of PSII in capsicum leaves in the presence of lincomycin obeys the law of reciprocity of irradiance and the duration of illumination, such that combinations of irradiance and duration that give the same photon exposure (mol incident photons m^{-2}) induce the same extent of photoinactivation (Chow et al. 2002), the decline in functional PSII was plotted against photon exposure for photoinhibitory irradiances ranging from 750 to 1300 μmol photons m^{-2} s^{-1} (Fig. 1). Two photoinhibition treatments were used. In one treatment, leaf segments were floated with the abaxial (lower) side in contact with the lincomycin solution,

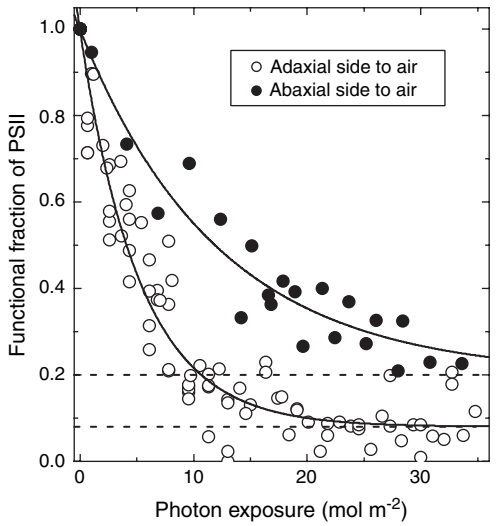

Fig. 1. Decrease in the functional fraction of photosystem II (PSII) with increase in photon exposure given to leaf segments illuminated with white light (750–1300 μmol photons m^{-2} s^{-1}) at 25°C in the presence of lincomycin. Leaf segments were floated with either the adaxial (upper) or abaxial (lower) side exposed to air, and were all illuminated on the adaxial side. Functional PSII was quantified by the oxygen yield per repetitive, single-turnover flash.

whereas the adaxial side was illuminated in air. The residual fraction of functional PSII was small, being only about 8% (Fig. 1). In the other treatment, the adaxial side was in contact with the lincomycin solution and illuminated, whereas the abaxial side was exposed to air; in this case, the residual fraction of functional PSII was about 20%.

A search for increased PSI cyclic electron flow after photoinactivation of PSII

How were the residual functional PSII complexes protected? Accompanying the progressive photoinactivation of PSII is an enhanced dissipation of excitation energy as heat, indicated by a shorter lifetime of Chl *a* fluorescence from excited states as measured in the presence of 30 μ*M* DCMU at −2°C and in low red light (140 μmol m^{-2} s^{-1}) (Matsubara and Chow 2004). These measurement conditions were chosen to produce minimal linear electron flow, thereby minimizing the ΔpH that might otherwise give rise to antenna quenching. It was hoped that reaction-center quenching of excitation energy could then be revealed by the shorter Chl *a* fluorescence lifetime observed after progressive photoinactivation of PSII (Matsubara and Chow 2004). However, enhanced cyclic electron flow around PSI that gives an increased ΔpH and greater antenna quenching could in principle occur even when PSII was photoinactivated. We wondered, therefore, if cyclic electron flow around PSI occurred under these conditions. If so, a progressively shorter Chl *a* fluorescence lifetime, as observed *after* increasing duration of photoinhibition, should be accompanied by a higher rate of cyclic electron flow around PSI under the same measurement conditions (30 μ*M* DCMU, −2°C and low red light).

To monitor any potential increase in cyclic electron flow around PSI, we measured the flux of electrons arriving at the donor side of PSI in the presence of DCMU, by measuring an absorbance change associated with the formation of the oxidized $P700^+$. After a photoinhibition treatment, a leaf segment was vacuum infiltrated with 30 μ*M* DCMU. First, we obtained the maximum signal corresponding to the complete photooxidation of P700. In the example in Fig. 2A, the bulk of P700 was first photooxidized by continuous far-red light. A xenon flash was then added to momentarily photooxidize the remaining P700. The peak value after the flash represents the entire content of photooxidizable P700, and was used to normalize the signal induced in the same leaf segment in the same geometry by low red light alone, giving the fraction of photooxidized P700 in the steady state during illumination.

The latter signal was obtained to derive the steady-state electron flux as illustrated by a particular example in Fig. 2B. A leaf segment (in this particular case, one that had been photoinhibited at 900 μmol photons m^{-2} $s^{-1}$for 5 min) was illuminated with low red light (120 μmol photons m^{-2} s^{-1}) in the presence of 30 μ*M* DCMU and at −2°C until a near steady state was reached. At steady state, the rate of photooxidation of P700 was balanced by the rate of reduction by an electron flux, including any cyclic flux. On abruptly turning off the red light, the initial rate of rereduction of $P700^+$ would be equal to the electron flux in the steady state. Therefore, our objective was to determine the initial rate of rereduction of $P700^+$ on abruptly turning off the red light. The rereduction kinetics were well fitted by the sum of two exponential decays (Fig. 2C), with amplitudes A_1 and A_2, rate coefficients k_1 and k_2, and the total initial flux of rereduction (hereafter termed "total electron flux") given by $A_1k_1 + A_2k_2$.

During the photoinhibition treatment at 25°C in the presence of lincomycin for up to 300 min, leaf segments were sampled for measurement of total electron flux in the presence of 30 μ*M* DCMU and red light (120 μmol photons m^{-2} s^{-1}) and at −2°C, conditions similar to those used by used by Matsubara and Chow (2004) to observe the shorter Chl *a* fluorescence lifetimes. There was no increase in the total normalized electron flux with photoinhibition time (Fig. 3A); instead, the already low total

electron flux ($\leq$1.2 s^{-1}, mainly because of incomplete inhibition of PSII in leaf tissue by 30 μM DCMU) was further decreased.

Linear electron fluxes

We next measured the DCMU-sensitive, linear electron flux (the difference between + and − DCMU) under the same conditions as used *during* the photoinhibition treatment, viz., in the presence of lincomycin, at 25°C and in the presence of strong white light (900 μmol photons m^{-2} s^{-1}) (Fig. 3B). Because 30 μM DCMU did not completely inhibit PSII activity in leaf tissue, the concentration was increased to 100 μM. The DCMU-sensitive electron flux was obtained in either the presence or absence of MV (300 μM), which is usually added to divert electrons to oxygen and abolish PSI cyclic electron transport. The normalized DCMU-sensitive electron flux (Fig. 3B) was much higher than in Fig. 3A because of the higher irradiance and warmer temperature, reaching ca 14 s^{-1} in control samples. It even increased slightly after about 1 h illumination, consistent with a slight increase in the capacity for O_2 evolution (Lee et al. 1999), perhaps because of light activation of enzymes. Even after 300 min of photoinhibition, a normalized DCMU-sensitive electron flux of about 5 s^{-1} remained when measured at 900 μmol photons m^{-2} s^{-1} whether MV was present or not (Fig. 3B). That is, the remaining functional PSII complexes were responsible for a substantial linear electron flux, even after 300 min photoinhibition. When DCMU was omitted during the measurement at 900 μmol photons m^{-2} s^{-1}, we measured the total electron flux to $P700^+$, including any cyclic flux that might have been present. Significantly, there was little or no effect of MV (Fig. 3C), suggesting that there was little or no cyclic electron flow around PSI under those conditions.

Because the remaining functional PSII complexes were able to sustain a substantial linear electron flux, we hypothesized that the linear electron flux contributed to the photoprotection of the residual functional PSII by establishing a trans-thylakoid ΔpH, the lumen acidification in turn giving rise to antenna quenching (Demmig-Adams and Adams 1992, Govindjee 2002, Horton et al. 1996, Niyogi 2000). If so, given the different observed contents of residual functional PSII in leaf segments

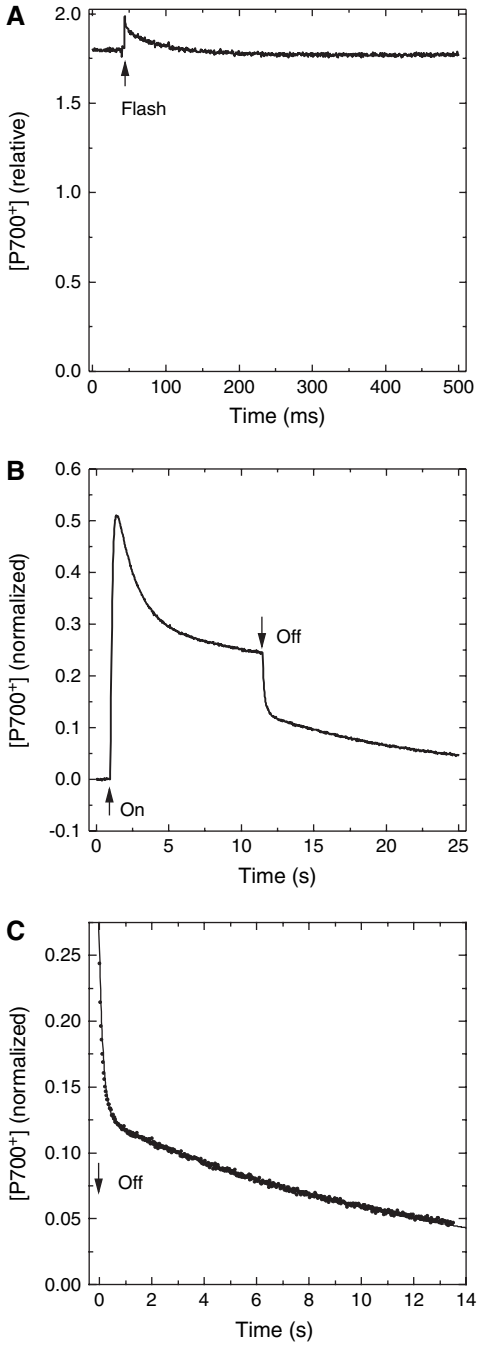

Fig. 2. A protocol for determining the redox kinetics of special Chl pair in the photosystem I reaction center (P700). (A) This particular leaf disk had been photoinhibited for 5 min (900 μmol photons m^{-2} s^{-1}) and was then illuminated with continuous far-red light (12 μmol photons m^{-2} s^{-1}) to photooxidize P700 to a steady state at −2°C in the presence of 30 μM 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea. A single-turnover flash was applied to momentarily photooxidize any remaining P700, yielding the total signal of photooxidizable P700. (B) Red light (120 μmol photons m^{-2} s^{-1}) alone was turned on to partially photooxidize P700 to near steady state, and then abruptly turned off to examine the rereduction kinetics of oxidized P700 ($P700^+$). The signals were normalized to the total signal from (A), obtained in the same geometry and for the same leaf segment. (C) The rereduction kinetics of the $P700^+$ signal from (B) were fitted as the sum of two exponential decays (solid line), with amplitudes A_1 and A_2, rate coefficients k_1 and k_2, and the total initial normalized flux of rereduction given by $A_1k_1 + A_2k_2$.

floating with the upper or lower leaf surface exposed to air while illuminated on the same adaxial side (Fig. 1), we anticipated that different linear electron fluxes should occur in the leaf segments in the two treatments. Indeed, Fig. 4 shows that the ETR was higher in leaf segments floated with the abaxial side exposed to air, compared with those floated with the adaxial side exposed to air; in both cases, the leaf segments were illuminated on the adaxial side.

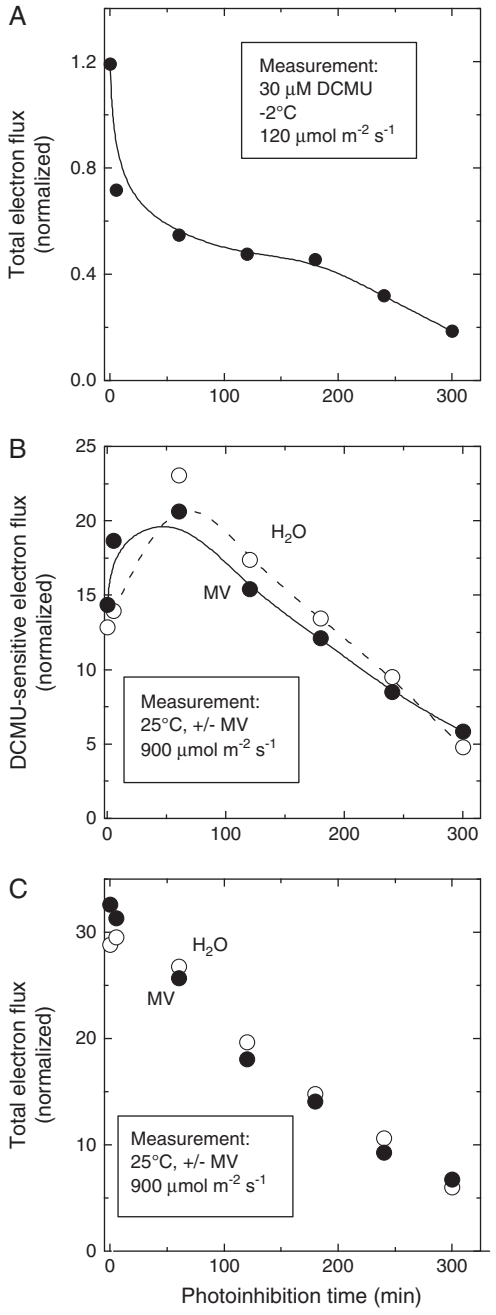

Fig. 3. Changes in the normalized total electron flux to oxidized special Chl pair in the photosystem I reaction center as a function of photoinhibition time. (A) Measurement at −2°C with red light (120 μmol photons m^{-2} s^{-1}). Leaf disks (floated on 1 m*M* lincomycin with the adaxial side to air) were photoinhibited at 900 μmol photons m^{-2} s^{-1}, and vacuum infiltrated with 30 μ*M* 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), kept in darkness at −2°C for 20 min and then preilluminated for 10 min with the red light before measurement. Each point is the mean for three leaf segments. (B) Measurement at 25°C with white light (900 μmol photons m^{-2} s^{-1}). Leaf disks (floated on 1 m*M* lincomycin with the adaxial side to air) were photoinhibited at 900 μmol photons m^{-2} s^{-1}, and vacuum infiltrated with (1) H_2O, (2) H_2O + 300 μ*M* methyl viologen (MV), (3) 100 μ*M* DCMU or (4) 100 μ*M* DCMU + 300 μ*M* MV, kept in darkness for 20 min and then preilluminated with the white light for 5 min before measurement at 25°C. For simplicity, the DCMU-sensitive electron flux in either the presence or absence of MV is plotted. Each point is the mean for two leaf segments. (C) The total electron flux determined in the absence (H_2O) or presence of MV, without any DCMU. Other conditions are as for (B).

The critical role of residual functional PSII complexes in recovery

Next, we tested the hypothesis that the small residual fraction of functional PSII complexes was critical for the recovery of the photoinactivated PSII. Leaf segments were photoinhibited at ca 7°C to avoid the use of lincomycin, until about 20% of functional PSII remained, and then allowed to recover in low light and at 25°C, either in the presence or absence of DCMU. Fig. 5 shows that DCMU completely inhibited the recovery, whereas recovery was substantial in 3 h in the absence of DCMU.

Discussion

A global analysis of Chl *a* fluorescence lifetime distributions revealed the presence of at least two populations of photoinactivated PSII centers in capsicum leaf segments (Matsubara and Chow 2004). One population (lifetime 1.25 ns) developed first at the expense of a 2.25-ns population of functional PSII complexes. Later, a population with a 0.58-ns lifetime arose, whereas the functional population continued to decline (Matsubara and Chow 2004). The 0.58-ns population was interpreted as strongly quenching photoinactivated PSII complexes that serve to protect a residual population of functional PSII (Matsubara and Chow 2004). Specifically, the strong quenching could arise from efficient thermal dissipation in photoinactivated PSII reaction centers. Although this hypothesis remains an attractive explanation for the maintenance of residual functional PSII complexes (Fig. 1), we needed to test whether additional quenching of excitation energy occurred in the light-harvesting

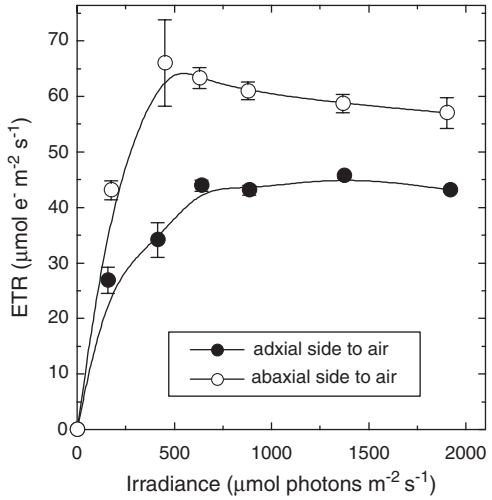

Fig. 4. The rate of electron transport through photosystem II (ETR) in control leaf disks as a function of measurement irradiance. Control leaf disks were floated on water, with either the adaxial (upper) or abaxial (lower) side in contact with water. The adaxial side was illuminated in both cases. ETR was calculated as described in Materials and methods. Each point is the mean (±standard error of the mean) of four leaf segments.

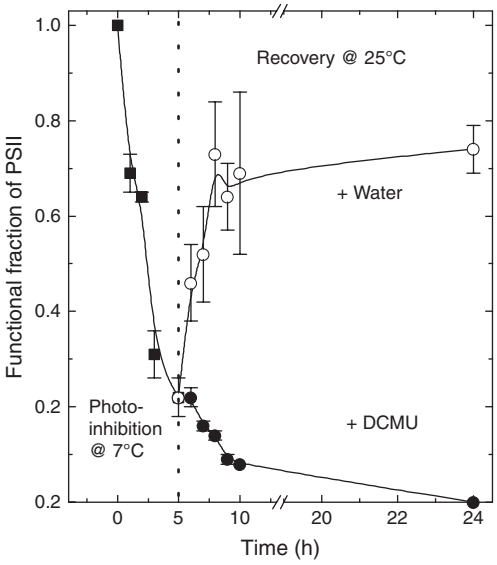

Fig. 5. The effect of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) on recovery of photosystem II (PSII) from photoinactivation. Small leaf segments (ca 5 × 5 mm^2) were photoinhibited (1000 μmol photons m^{-2} s^{-1}) at ca 7°C for 5 h in the absence of lincomycin for subsequent examination of recovery. Photoinhibited leaf disks were first floated on water (±50 μ*M* DCMU, adaxial side to air) in darkness for 30 min with gentle stirring, and allowed to recover under low light (20 μmol photons m^{-2} s^{-1}) at 25°C. The functional fraction of PSII was quantified by the Chl *a* fluorescence parameter $1/F_o - 1/F_m$ (see Materials and methods).

antennae surrounding neighboring functional and non-functional PSII centers.

Antenna quenching does not account for the strong quenching in photoinactivated PSII complexes measured when little or no PSII activity was permitted

Under measurement conditions (30 μ*M* DCMU, low red light at 120 μmol photons m^{-2} s^{-1} and at −2°C), the total flux of electrons flowing to $P700^+$ declined rather than increased with photoinhibition time (Fig. 3A). This observed decline in total electron flux cannot provide an expected increase of the trans-thylakoid ΔpH, which would be required to account for any increase in antenna quenching being responsible for a shorter lifetime of Chl *a* fluorescence measured under similar conditions (Matsubara and Chow 2004).

The decline in the observed total electron flux with photoinhibition time (Fig. 3A) further suggests that no increase in PSI cyclic electron flow occurred after photoinhibition when measured in the presence of 30 μ*M* DCMU and red light at 120 μmol photons m^{-2} s^{-1}, and at −2°C. Consistent with this conclusion, cyclic electron flow via PSI as monitored photoacoustically, although persistent in the presence of DCMU, was no greater in photoinactivated *Chlamydomonas* cells than in control cells (Canaani et al. 1989). Further, although cyclic electron transport via PSI as monitored photoacoustically in the *absence* of DCMU was stimulated after photoinhibition of maple seedlings, DCMU completely abolished these photoacoustic signals representing PSI and PSII activities (Veeranjaneyulu et al. 1998). Thus, there is little evidence for a postphotoinhibition increase in cyclic electron flow that can be measured in the presence of DCMU. Therefore, we conclude that the strong quenching of excitation energy, observed in the presence of DCMU, low red light and at −2°C, is likely to be caused by reaction-center dissipation rather than antenna quenching.

Linear electron transport, however, contributes to antenna quenching under photoinhibition conditions

The total flux of linear electron flow through PSI is given by the difference between the absence and presence of DCMU. This DCMU-sensitive component was observed with strong white light (900 μmol photons m^{-2} s^{-1}) at 25°C (Fig. 3B), conditions that are similar to those *during* photoinhibition. Linear electron flow was still substantial after 300 min of photoinhibition in the presence of lincomycin, the normalized flux being 5 s^{-1}, compared with 14 s^{-1} after only 5 min strong light. The linear electron flux could conceivably be a major contributor to

a trans-thylakoid ΔpH required for antenna quenching (Demmig-Adams and Adams 1992, Govindjee 2002, Horton et al. 1996, Niyogi 2000). The contribution of ΔpH-dependent antenna quenching to the photoprotection of residual functional PSII population explains an earlier observation that the residual population fell to a very low level after photoinhibition of leaf segments in the combined presence of nigericin (an uncoupler) and lincomycin (Lee et al. 2002). We propose that such antenna quenching, associated with a ΔpH established by linear electron flow via the residual functional PSII complexes, could operate in parallel with reaction-center quenching associated with photoinactivated PSII complexes during photoinhibition.

Interestingly, there was little or no effect of MV on the total electron flux to P700 whether DCMU was absent (Fig. 3C) or present (data not shown). We interpret this to mean that any cyclic electron flux was small in comparison with the linear electron flux. The measurement of electron fluxes used a 5-min preillumination (900 μmol photons m^{-2} s^{-1}) followed by repetitive 2-s pulses of the same strong white light. Repeated illumination could have induced a transition from cyclic to linear mode of electron flow, as proposed by Joliot and Joliot (2005); this may be the reason why we did not observe any significant cyclic electron flow with white light illumination. If cyclic electron flow could be increased somehow, energy-dependent processes such as antenna quenching should be enhanced. For example, energy-dependent uptake of cytoplasmically synthesized polypeptides into chloroplasts is stimulated by cyclic electron flow around PSI in the presence of dithiothreitol, so much so that linear electron flow can be inhibited without any effect on the uptake (Grossman et al. 1980). In our system without the addition of exogenous reductants, however, the rate of cyclic electron transport was minimal.

If linear electron transport associated with residual functional PSII complexes contributed to their own photoprotection, we expected that stimulation of linear electron flow would enhance the photoprotection. Because we observed that the stomatal density was 143 ± 8 mm^{-2} (±SE, $n = 18$ leaf segments) on the abaxial surface, and 59 ± 4 mm^{-2} (±SE, $n = 24$ leaf segments) on the adaxial surface of capsicum leaves, we expected to stimulate gas exchange, and therefore electron flow, by floating leaf segments with the abaxial side facing air compared with the other orientation, as was indeed observed (Fig. 4). Consistent with this observation, the size of the residual functional population of PSII was significantly increased compared with floating leaf segments with the adaxial side in contact with air and illuminated (Fig. 1). Exposing the stomate-rich abaxial side to air would enable both CO_2 and O_2 to diffuse into the tissue more readily. An increased supply of CO_2 would increase photosynthetic rate, leaving a smaller fraction of excess excitation energy that may cause photoinactivation.

PSII, comprising a reaction-center dimer, core components and various light-harvesting Chl *a*/*b*–protein complexes (LHCII), is located in the intricate macrostructure of stacked granal domains. The LHCII–PSII supercomplexes associate with further LHCII trimers to form macrodomains (Dekker and Boekema 2005). Results reported here and earlier (Anderson and Aro 1994) suggest that connectivity between functional and non-functional PSII persists on prolonged illumination. The summary scheme in Fig. 6 depicts that the survival of a small residual functional PSII population during prolonged illumination in the absence of repair is probably because of (1) transfer of excitation energy (solid arrows) from functional PSII to photoinactivated PSII complexes that are capable of efficient thermal dissipation (medium-sized hollow arrows) of the energy in the reaction center and (2) enhanced quenching of excitation energy (largest hollow arrow) in a common antenna (dotted envelope), facilitated by a ΔpH, which is sustained by linear electron transport associated with the

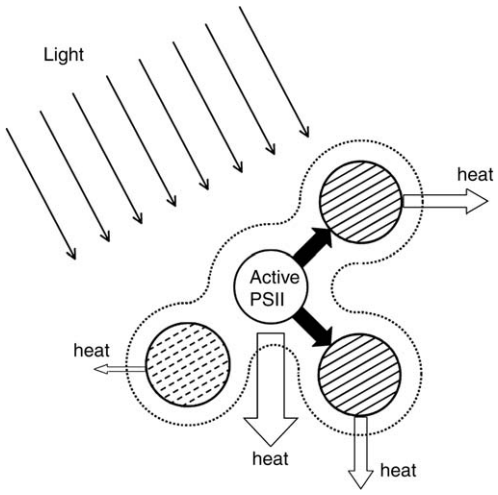

Fig. 6. A diagram showing three types of photosystem II (PSII) units under prolonged illumination of leaf segments, connected via their antennae (dashed envelope). A functional PSII (non-hatched circle) passes its excitation energy to, and is photoprotected by, strongly dissipating, photoinactivated PSII centers (hatched with solid lines), whereas an intermediate, weakly dissipating type of photoinactivated PSII (hatched with dashed lines) is presumed to have a limited dissipation of excitation energy. In strong light, even when most of PS II centers are photoinactivated, high-energy-state quenching in the antennae connecting both functional and non-functional PSII also occurs (depicted by the largest arrow), induced by a trans-thylakoid pH gradient that is sustained by linear electron flow through the residual functional PSII. Modified from Matsubara and Chow (2004).

residual active PSII. Further, some thermal dissipation (smallest hollow arrow) may occur in weakly dissipating non-functional PSII reaction centers.

The small residual functional PSII complexes are critical for recovery of photoinactivated PSII

If photoinactivated PSII complexes help to protect their residual functional neighbors under prolonged illumination, what role do the functional PSII complexes play in the recovery of photoinactivated complexes in weaker light? Fig. 5 shows that when PSII was severely photoinactivated at a chilling temperature (in the absence of lincomycin), recovery was easily observed, but only if the residual functional PSII complexes were not inhibited with DCMU during recovery. That is, the residual functional PSII complexes were critical for recovery of photoinactivated PSII units. Thus, photoinactivated PSII and functional PSII play a mutually beneficial role in each other's ultimate survival.

Linear electron transport sustained by the residual functional PSII complexes could be important for recovery of photoinactivated PSII for several reasons. It could provide the adenosine triphosphate (ATP) necessary for biochemical reactions required to replace photodamaged D1 protein by a newly synthesized D1 (Mattoo et al. 1984, Taniguchi et al. 1993) or for the uptake of cytoplasmically synthesized polypeptides into the chloroplast (Grossman et al. 1980). Indeed, recovery that is normally negligible, though non-zero, in darkness (He and Chow 2003) could be partially induced by floating photoinhibited leaf segments on a 50 m*M* solution of ATP in the dark (H.-Y. Lee and W.S. Chow, unpublished), just as the transport of polypeptides into the chloroplast can be induced by ATP in the dark (Grossman et al. 1980). Linear electron transport could also provide reducing equivalents necessary for the translational regulation of D1 protein (Kuroda et al. 1996, Trebitsh and Danon 2001). Further, the requirement of light for the efficient translation elongation and subsequent integration of the D1-protein into PSII (van Wijk and Eichacker 1996) could be mediated through linear electron flow. Therefore, the residual PSII activity is critical for repair of photoinactivated PSII.

In conclusion, the residual functional PSII complexes were photoprotected by (1) quenching of excitation in photoinactivated PSII reaction centers, and (2) antenna quenching, which was sustained by linear electron transport in functional PSII, but apparently not by any substantial contribution from cyclic electron flow around PSI. In turn, the residual functional PSII complexes were critical for the subsequent recovery of photoinactivated PSII in weaker light. Although photoinactivation of PSII is generally regarded as a detrimental process, a novel photoprotective strategy aids the survival of the leaf in extreme environments.

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