High-irradiance stress and photochemical activities of photosystems 1 and 2 *in vivo*

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Abstract

High-irradiance (HI) stress induced changes in the photosynthetic energy storage (ES) of photosystems 1 (ES_{PS1}) and 2 (ES_{PS2}) were studied with 650 nm modulated radiation in intact sugar maple (*Acer saccharum* Marsh.) leaves. HI-treatment (420 W m^{-2}, 1 h) caused an inhibition of about 40 % in ES_{PS2} and an enhancement of about 60 % in ES_{PS1}. The rate of PS1 cyclic electron transport, measured with 705 nm modulated radiation, also increased in HI-treated leaves. There was a clear state 1-state 2 transition in HI-treated leaves. ES_{PS1} increased significantly and ES_{PS2} decreased drastically in leaves preadapted to state 1 after HI (600 W m^{-2}, 30 min) treatment. Thus, the increase in PS1 activity observed immediately after HI-treatment in leaves preadapted to state 1 can be due to the coupling of LHC2 to PS1 during the HI-treatment. Further, the dissociation of LHC2 from PS2 during the HI-treatment resulted in apparently (about 15 %) greater inhibition than the "true" inhibition of PS2 activity. The presence of LHC2 with PS2 (state 1) at the time of HI-treatment caused no additional damage to PS2 or its coupling to PS1 offered no apparent protection to the photosynthetic apparatus.

Additional key words: *Acer saccharum*; light-harvesting complex 2; photoacoustic spectroscopy.

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Abbreviations: cyt: cytochrome; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea; ES: energy storage; ES_T: energy storage of PS1 and PS2; ES_{PS1}: energy storage of PS1 (measured at 650 nm); ES_{PS1-cy}: energy storage of cyclic electron transport around PS1 (measured at 705 nm); ES_{PS2}: energy storage of PS2 (measured at 650 nm); F_0: initial level of fluorescence; F_m: maximum level of fluorescence; F_v: variable fluorescence; HI: high-irradiance; LHC2: light-harvesting complex of PS2; li-ES_{PS1}: PS2-dependent PS1 energy storage (measured at 705 nm); PA: photoacoustic; PQ: plastoquinone; PS: photosystem; WL: "white light".
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Introduction

Photosynthetically active radiation drives the photochemical reactions of PS1 and PS2 in oxygen evolving photosynthetic organisms. Nevertheless, excessive radiant energy inhibits photosynthesis; this phenomenon is termed photoinhibition (Critchley 1988, Aro *et al.* 1993, Long *et al.* 1994). The photoinhibition occurs at thylakoid level, particularly at PS2 (Kyle *et al.* 1984, Cleland *et al.* 1986, Critchley 1988, Eckert *et al.* 1991). Several investigators view photoinhibition of photosynthesis as a process of stress-induced damage to PS2. This view is based on the fact that, as a consequence of photoinhibition, the D1 protein of PS2 reaction center becomes degraded (Kyle *et al.* 1984, Prasil *et al.* 1992, Rintamäki *et al.* 1995). But some recent reports suggest that photoinhibition, first of all, results from the formation of photochemically inactive PS2 centers (without any degradation of the D1 protein), which convert the excitation energy into heat. This down regulation of PS2 and thermal dissipation is considered as a protective mechanism against HI-stress (Cleland *et al.* 1986, Krause 1988, Aro *et al.* 1993, van Wijk and van Hasselt 1993, Gilmore and Björkman 1994). The photoinactivation and impairement of electron transport occur at the acceptor and donor sides of PS2, although inactivation of the acceptor side may be the main mechanism for the impairement of electron transport (Eckert *et al.* 1991, Aro *et al.* 1993). This evidence comes largely from the *in vitro* studies. But the mechanism of photoinhibition *in vivo* may be more complicated, and the dominating mechanism of inactivation *in vivo* is not clear.

PS1 inhibition is also reported under HI-stress (Harvey and Bishop 1978, Tyystjärvi *et al.* 1989), but PS1 is relatively less susceptible than PS2 to this stress (Critchley 1981, Tyystjärvi *et al.* 1989). These studies were made on algal cells or on chloroplasts isolated from HI-treated leaves in the presence of artificial electron donors and acceptors (Critchley 1981, Satoh and Fork 1982, Tyystjärvi *et al.* 1989). PA spectroscopy shows that PS1 is resistant to HI-stress in *Chlamydomonas reinhardtii* cells and pea leaves (Canaani *et al.* 1989, Havaux and Eyletters 1991). Information on the nature of HI-stress induced changes in the relative activities of PS1 and PS2 *in vivo* in higher plants is limited. Therefore, one of the objectives of the present study is to contribute to their understanding.

LHC2 migration between the photosystems causes redistribution of absorbed energy between PS1 and PS2 (Fork and Satoh 1986, Williams and Allen 1987). As demonstrated, PS1 and PS2 activities can change significantly due to a change in their absorption cross-section brought by the migration of LHC2 between them (Veeranjaneyulu *et al.* 1991a,b, Veeranjaneyulu and Leblanc 1994). Its phosphorylation and dissociation from PS2 is considered as a protective mechanism against HI-stress (Horton and Lee 1985, Öquist 1988). But there is no convincing experimental evidence to support this concept. Besides this, there are also conflicting reports on the activity of thylakoid protein kinase, which is responsible for LHC2 phosphorylation. Thylakoid protein kinase activity, regulated by redox state of PQ pool and cyt b_6f complex, is high under HI (Horton and Foyer 1983). In contrast to this, the HI-stress inactivates protein kinase (Schuster *et al.* 1986) and affects the state 1-state 2 transitions in *C. reinhardtii* cells (Canaani *et al.* 1989). Hence, another

objective of this study was to learn the occurrence of state 1-state 2 transitions, and to understand the extent of protection offered, if any, to the photosynthetic apparatus by the dissociation of LHC2 from PS2 in the HI-treated leaves.

In the literature, there is little information on the magnitude of LHC2-decoupling induced change in PS2 activity in HI-treated leaves. A reduction in absorption cross-section of PS2 due to LHC2 dissociation (Horton and Lee 1985, Öquist 1985) can cause a significant decrease in PS2 activity (Veeranjaneyulu *et al.* 1991a,b, Veeranjaneyulu and Leblanc 1994). Hence, we felt that it was necessary to isolate the magnitude of LHC2-decoupling induced decrease in PS2 activity in order to understand the "real" damage to PS2 caused by HI-treatment.

In the present study, we used photoacoustic methodology to follow the changes in relative activities of PS1 and PS2 by measuring the photosynthetic energy storage in HI-treated leaves.

Materials and methods

Plants: Three months-old seedlings of sugar maple (*Acer saccharum* Marsh.) were raised in pots (10×10 cm) containing a mixture of organic soil, peat moss, vermiculite, and sand (4 : 1 : 1 : 0.5, v/v). They were maintained in a growth room with a photoperiod of 14 h, irradiance of 12 W m^{-2} (sodium arc-tube lamp, *Phillips Electronics*, Scarborough, ON, Canada), temperature of 23 ± 2 °C, and relative humidity from 60 to 70 %. The seedlings were watered twice a week with tap water. Biweekly, N-P-K fertilizer (*11-41-8, Plant Products Co.*, Brompton, ON, Canada) was added at a concentration of 2 kg m^{-3}. Seedlings with 3 to 5 pairs of leaves were used for HI-treatments. Fully expanded, mature (between 20 to 30 d after expansion), and dark green leaves were used.

Photoinhibitory treatment: Measurements were made on the same leaf before and after HI treatment, to avoid variations among different plants. Before HI-stress, an 18 mm-diameter disk was punched from one half of the leaf avoiding the major veins for control sample. HI-treatment was given by exposing perpendicularly the adaxial surface of the other half of the leaf (attached to the plant) to the strong "white light" (WL) from a projector lamp (model *EKE, Sylvania*) filtered through a 10 cm layer of water. HI-treatment was at a photon flux density of 420 W m^{-2} for 1 h, unless otherwise stated in the text. Irradiance at the surface of the leaf was measured with a light-meter (model *LI-189, LI-COR*, Lincoln, NE, USA). After the treatment, another disk was taken from the light-exposed region of the leaf. For state 1-state 2 transition experiments, measurements were made on the same leaf disk before (control) and after HI-treatment. The treatment was given by exposing the leaf disk placed on a moist filter paper to the radiation filtered through a 10 cm layer of water.

Photoacoustic measurements were made in a lab-built PA spectrometer as described by Veeranjaneyulu *et al.* (1991a). Radiant beam from a xenon arc lamp (*Canrad-Hanovia*, Newark, NJ, USA) coupled to a monochromator (model *GM251-2, Schoeffel Instrument Co.*, Westwood, NJ, USA) was modulated by a mechanical

chopper (model *218F, Bentham*, Ithaca, NY, USA), and was reflected by a mirror on the adaxial surface of the leaf disk placed in the PA cell. Continuous background WL (from a tungsten-halogen lamp, model *EKE, Sylvania*) was directed on the sample *via* a fiber optic light guide, in order to saturate modulated photochemistry. PA signals were detected by a microphone (model *1972-9600, General Radio*, Bolton, MA, USA), amplified, analyzed by a lock-in amplifier (model *393, Ithaco-Dynatrac*, Ithaca, NY, USA), and displayed on a chart recorder.

Photoacoustic methodology allows the determination of heat emission and O_2 exchange from a leaf disk irradiated with modulated radiation. Heat emission alone can be recorded at high modulation frequencies where there is no O_2 contribution to the signal. This technique permits to estimate photon utilization efficiency of photosynthetic apparatus (Malkin and Cahen 1974, Malkin and Canaani 1994). By measuring heat emission in the presence and in the absence of saturating irradiance of continuous background WL (saturating WL closes all photosynthetic reaction centers, resulting in the release of all absorbed modulated radiation as heat and radiation), the amplitude of modulated photochemistry which represents photosynthetic energy storage (ES) can be determined. This ES represents the part of absorbed modulated radiation which is stored in the intermediates of electron transport chain.

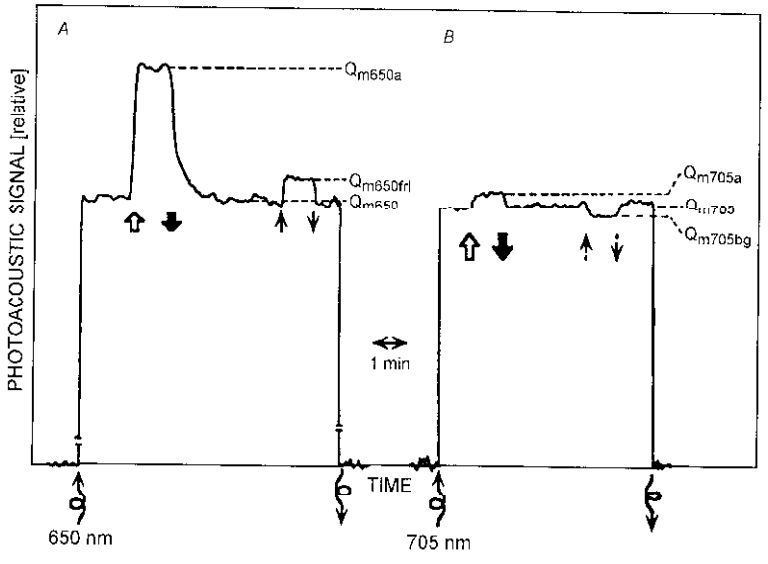

Fig. 1. Photoacoustic signal from a sugar maple leaf recorded with 650 (*A*) or 705 nm (B) modulated radiation. (↑, ↓) modulated radiation on and off, respectively. 650 nm, 2 W m^{-2}; 705 nm, 2.4 W m^{-2}; frequency: 100 Hz. (⇧, ⇩) background "white light" (180 W m^{-2}) on and off, respectively. (↑, ↓) background far-red radiation (λ > 715 nm, 62 W m^{-2}) on and off, respectively. (↑, ↓) background blue-green radiation (13 W m^{-2}) on and off, respectively.

Energy storage of both photosystems together (ES_T) was determined by recording the signal with 650 nm modulated radiation at 100 Hz, in the presence (Q_{m650a}) and in the absence (Q_{m650}) of continuous background saturating irradiance by WL

(Fig. 1*A*). At this frequency, O_2 contribution to the PA signal is negligible, and the measured ES_T is comparable to that measured at 500 Hz in sugar maple leaves.

$$ES_T = ES_{PS1} + ES_{PS2} = \frac{Q_{m650a} - Q_{m650}}{Q_{m650a}} \times 100 \quad (1)$$

PS1 energy storage (ES_{PS1}) was measured by using continuous background far-red radiation ($\lambda > 715$ nm) to saturate PS1 photochemistry (Veeranjaneyulu *et al.* 1991b). The background far-red radiation was provided by placing a series of cut-off filters *GG427, OG560, RG645,* and *RG715* in the path of background WL. The difference in the amplitude of signal in the presence ($Q_{m650frl}$) and absence (Q_{m650}) of continuous background far-red radiation (Fig. 1*A*) was taken as the energy storage of PS1 (Veeranjaneyulu *et al.* 1991a).

$$ES_{PS1} = \frac{Q_{m650frl} - Q_{m650}}{Q_{m650a}} \times 100 \quad (2)$$

PS2 energy storage (ES_{PS2}) was derived by subtracting ES_{PS1} from ES_T:

$$ES_{PS2} = ES_T - ES_{PS1} \quad (3)$$

Energy storage during cyclic electron transport around PS1 ($ES_{PS1\text{-}cy}$) was determined by recording the signal with 705 nm modulated radiation (Fig. 1*B*) in the presence (Q_{m705a}) and absence (Q_{m705}) of continuous background WL (Canaani *et al.* 1989, Herbert *et al.* 1990):

$$ES_{PS1\text{-}cy} = \frac{Q_{m705a} - Q_{m705}}{Q_{m705a}} \times 100 \quad (4)$$

PS2-dependent PS1 activity (li-ES_{PS1}) was measured by recording the signal with 705 nm modulated radiation in the presence (Q_{m705bg}) and absence (Q_{m705}) of continuous background blue-green radiation. This background blue-green radiation was provided by placing a *Corning 4-96* filter (*Oriel Electro-optics*, Watford, UK, model *CS 4-96*) in the path of the background WL:

$$li\text{-}ES_{PS1} = \frac{Q_{m705} - Q_{m705bg}}{Q_{m705a}} \times 100 \quad (5)$$

Blue-green radiation was used as PS2 radiation, because of relatively low PS1 activity in the blue region of the spectrum between 440 and 500 nm (Veeranjaneyulu and Leblanc 1994). The background PS2 irradiance was selected to ensure the recording of maximum energy storage, and it was the same for the samples before and after HI-treatment.

Fluorescence measurements: For these measurements, the above described PA set up was slightly modified. A trifurcated light guide with one arm to deliver the modulated radiation, a second arm for the background blue-green radiation, and a third arm to collect the fluorescence, was used. Background blue-green radiation was provided by placing a *Corning* blue-green glass filter (*Oriel Electro-Optics*, Watford,

UK, model *CS 4-96*) in the path of background WL to avoid the interference of red radiation with fluorescence measurements. Fluorescence detection system consisted of a 680 nm band-pass interference filter (*Ditric*, Hudson, MA, USA) and a photodetector (*United Detector Technology*, Santa Monica, CA, USA, model *PIN-10D*). The signal was amplified, and analyzed by a lock-in amplifier (*Ithaco-Dynatrac*, Ithaca, NY, USA, model *393*) with a time constant of 400 ms. Initial fluorescence (F_0) was measured with a weak modulated radiation (430 nm, 83 mW m^{-2}, 100 Hz), and fluorescence maximum (F_m) was determined by adding a pulse of blue-green radiation (180 W m^{-2}). The F_0 level was checked by observing that background far-red radiation had no quenching effect. The difference between F_m and F_0 was taken as variable fluorescence (F_v).

State 1-state 2 transitions were studied according to Canaani *et al.* (1984) as described by Veeranjaneyulu *et al.* (1991a). A leaf disk in the PA cell was driven to state 2 or state 1 by irradiation with 650 nm modulated radiation or by both 650 nm modulated radiation and background far-red radiation, respectively, for 25 min. ES_T, ES_{PS1}, and ES_{PS2} were studied as described above.

Results and discussion

Radiant energy absorbed by photosynthetic pigments is partly stored as chemical energy in various intermediates, and the rest is dissipated into the environment. This stored energy (ES_T), which represents the photochemical activities of PS1 and PS2, was completely inhibited by DCMU (50 μM) treatment (values not shown). Hence, both ES_{PS1} and ES_{PS2} are sensitive to DCMU.

Table 1. Effect of high-irradiance (HI) treatment on ES_T, ES_{PS1}, ES_{PS2}, ES_{PS2}/ES_{PS1}, $ES_{PS1\text{-}cy}$, and li-ES_{PS1} of intact sugar maple leaves. Values [% of absorbed energy] are means of six replicates ± SD. Figures in parentheses indicate % control.

	Control	HI-treated
ES_T	32.2 ± 4.3	23.6 ± 3.9 (73 ± 10)
ES_{PS1}	3.5 ± 0.7	5.6 ± 1.8 (158 ± 37)
ES_{PS2}	28.7 ± 4.4	18.0 ± 5.1 (63 ± 16)
ES_{PS2}/ES_{PS1}	8.2 ± 2.2	3.9 ± 2.8
$ES_{PS1\text{-}cy}$	4.4 ± 2.0	6.6 ± 1.3 (152 ± 34)
li-ES_{PS1}	7.1 ± 2.5	8.5 ± 2.5 (122 ± 26)

In general, ES_{PS1} is low when compared to ES_{PS2}, possibly due to an imbalance in 650 nm excitation distribution in favor of PS2, a situation normally observed under state 1 conditions in sugar maple seedlings (Table 1). Under steady state, the ratio of ES_{PS2}/ES_{PS1}, which varies between state 1 and state 2, is closer to the one observed under state 1 conditions. PS1 may be a limiting factor to the linear electron transport from PS2 in sugar maple seedlings. Such a condition may arise if one considers the stoichiometry of PS2 to PS1 is greater than 1. These stoichiometric values vary with

growth conditions and plant type (Jursinic and Dennenberg 1989). This will be explained further by comparing our measurements with 705 nm modulated radiation in control and HI-treated sugar maple leaves.

We followed the response of photochemical activity to saturating irradiance in sugar maple leaves by plotting the normalized difference of PA signal between Q_{m650} and Q_{m650a} against varying irradiances by background continuous WL (Fig. 2). As mentioned earlier, only at saturating background WL irradiance, all the reaction centers of PS1 and PS2 are closed and the absorbed radiant energy is released. This background irradiance can be considered as saturating. For sugar maple seedlings, it is about 180 W m^{-2} (Fig. 2). Any irradiance higher than this is an excess to the photochemistry of the leaf which can inhibit photosynthesis.

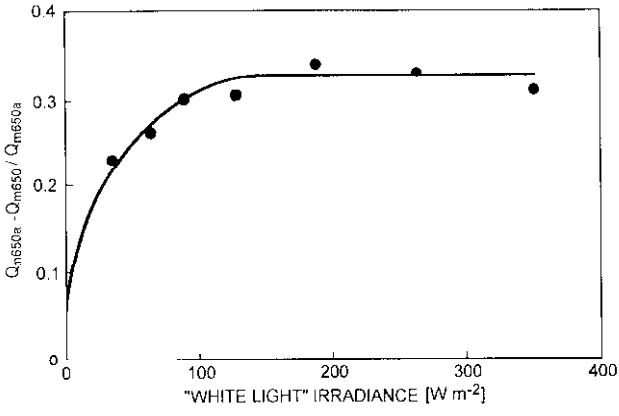

Fig. 2. A plot between $Q_{m650a} - Q_{m650}/Q_{m650a}$ and background "white light" irradiance in sugar maple leaves. Other conditions are as in Fig. 1*A*.

HI-treatment for 60 min caused a decrease of about 27 and 37 % in ES_T and ES_{PS2}, respectively (Table 1). In contrast to this, it caused an increase in ES_{PS1} by about 58 %. These observations of *in vivo* PS1 and PS2 activities indicate that PS2 is specifically inhibited, and PS1 is enhanced by HI-treatment.

We also measured energy storage with 705 nm modulated radiation, which is preferentially absorbed by PS1. As there is little, if any, electron transport from PS2 to PS1, when excited with 705 nm modulated radiation, energy storage calculated from the difference between the signal in the presence and in the absence of background WL ($ES_{PS1\text{-}cy}$) reflects the photochemical products formed during cyclic electron transport (Canaani *et al.* 1989, Herbert *et al.* 1990, Malkin *et al.* 1990, Malkin and Canaani 1994). This $ES_{PS1\text{-}cy}$ increased by about 52 % in HI-treated sugar maple leaves. Cyclic activity around PS1 is resistant to HI-treatment in *Chlamydomonas* cells (Canaani *et al.* 1989), and it increases transitorily during the first 20 min of treatment in pea leaves (Havaux and Eyletters 1991).

The increase in cyclic electron transport may be an adaptive process to HI-stress in sugar maple plants. The physiological significance of cyclic electron transport around PS1 in C_3 plants is not clearly understood (Heber and Walker 1992). Cyclic electron flow around PS1 may be necessary for prevention of photodamage to chloroplasts (Ridley 1977, Ridley and Horton 1984). Thus, the enhanced cyclic flow around PS1

can dissipate the excitation from PS1 and generate ATP molecules required during repair processes. It can also produce a large trans-thylakoid proton gradient necessary for the conversion of violaxanthin to zeaxanthin in the xanthophyll cycle (Hager 1969) which is involved in heat dissipation during HI-stress (Demmig-Adams *et al.* 1989).

The PS2-dependent PS1 activity (li-ES_{PS1}), measured in the presence of background PS2 (blue-green) radiation, indicates the energy stored in the intermediates of the electron transport chain, both upstream and downstream of PS1. The li-ES_{PS1} also increased, despite an about 40 % decrease in ES_{PS2} (Table 1). Hence, the electron flow from PS2 may not be limiting to PS1 in these HI-treated leaves. This suggests that PS2 population may be larger than that of PS1 *in vivo*. In the downstream of PS1, electrons may be accepted by O_2 (Mehler reaction), and the O_2 photoreduction may be a protective mechanism to dissipate the excess excitation, if there is an efficient scavenging system for reactive species (Krause 1988, Wu *et al.* 1991).

Thus, the above experiments showed that there is a significant increase in PS1 activity and a drastic decrease in PS2 activity. Recently, we have demonstrated that PS1 activity can increase during transition from state 1 to state 2 due to the migration of phosphorylated LHC2 from PS2 to PS1 (Veeranjaneyulu *et al.* 1991a). As the LHC2 phosphorylation and its dissociation from PS2 can reduce the antenna size, it may be considered as a protective mechanism against HI-stress (Fork and Satoh 1986, Öquist 1988). Hence, the LHC2 coupling to PS1 may also enhance its activity. Thus, we feel that it is necessary to understand the PS1 activity in state 1 (PS1 devoid of mobile LHC2) in HI-treated leaves.

Table 2. Changes in ES_T, ES_{PS1}, ES_{PS2} in state 1 and state 2 before and after high-irradiance (HI; 600 W m^{-2}, 30 min) treatment in sugar maple leaves. HI-treatment was given to the leaf disk preadapted to state 1. Values are in % of absorbed energy. Means of four replicates ± SD.

	Before HI-treatment			After HI-treatment			
	state 2	state 1	state 1 - state 2	immediately state 2		state 1	state 1 - state 2
ES_T	35.7 ± 2.7	35.5 ± 2.7		22.7 ± 2.3	24.3 ± 1.2	23.9 ± 1.2	
ES_{PS1}	9.0 ± 1.9	3.4 ± 1.1	-5.6	5.4 ± 1.1	6.2 ± 0.4	2.5 ± 1.1	-3.7
ES_{PS2}	26.7 ± 2.1	32.1 ± 3.0	+5.4	17.2 ± 2.0	18.2 ± 1.1	21.4 ± 2.1	+3.2
ES_{PS2}/ES_{PS1}	2.98	9.36		3.17	2.94	8.41	

Table 2 summarizes the results on the LHC2 coupling and decoupling associated changes in PS1 and PS2 activities before and after HI-treatment in leaves preadapted to state 1. As demonstrated earlier, ES_T remained nearly the same both in state 1 and state 2 in leaves before HI-treatment. But, both ES_{PS1} and ES_{PS2} changed significantly during state transitions. The fraction of energy lost by PS1 during transition from state 2 to state 1 was nearly equal to that gained by PS2. This fraction of energy transfer between PS1 and PS2 was about 5.6 % of the absorbed radiant energy, which confirms our earlier observations.

Immediately after the HI-treatment, as expected, both ES_T and ES_{PS2} decreased drastically (Tables 2 and 3). But ES_{PS1} increased in the samples preadapted to state 1. The ratio of ES_{PS2} to ES_{PS1} also decreased, and was close to that in state 2 (Table 2). Further, when the leaf was driven to state 2, there was a marginal recovery in ES_{PS1} and ES_{PS2} with a little decrease in the ratio of ES_{PS2} to ES_{PS1}. Thus, the increase in ES_{PS1} observed immediately after HI-treatment (Tables 1 and 2) is possibly due to the migration of LHC2 from PS2 to PS1 during the treatment for the samples preadapted to state 1. In order to check the occurrence of state transition phenomenon, we drove the leaf to state 1, and we noticed a clear change in ES_{PS1} and ES_{PS2} (Table 2). The ratio of ES_{PS2} to ES_{PS1} was close to that observed in state 1. Thus, there was a clear state transition in HI-treated sugar maple leaves (Table 2).

A comparison of ES_{PS1} in state 1 (PS1 devoid of mobile LHC2) or in state 2 before and after HI-treatment (Table 2) showed a significant decrease in PS1 energy storage during HI-treatment. This is due to the fact that ES_T is sensitive to DCMU (an inhibitor of PS2 electron transport) treatment, and any decrease in PS2 electron transport rate can reduce ES_{PS1}. However, when one compares the ES_{PS1} in state 1 before HI-treatment with ES_{PS1} after HI-treatment, a significant increase in PS1 activity can be seen. This is further confirmed from the increased PS1 activity measured with 705 nm modulated radiation. Thus, the increase in PS1 activity can be due to the increased absorption cross section of PS1 population during the HI-treatment. The fraction of energy transferred between PS1 and PS2 decreased by about 34 % [(5·6-3·7)/5·6)] after the HI-treatment. This decrease was comparable to the decrease of ES_T in state 2 or state 1 (about 32 %).

The dissociation of LHC2 from PS2 was considered as a protective mechanism against HI-stress, as PS2 is reported to be the primary target (Fork and Satoh 1986, Öquist 1988). The present study demonstrates the coupling of LHC2 to PS1 (increase in ES_{PS1}), and increase in its absorption cross section area during the HI-treatment. Thus, it may be asked whether dissociation of LHC2 from PS2 or its association with PS1 is a protective mechanism. In order to answer this, we exposed state 1- or state 2-preadapted leaves to HI, and followed the changes in ES_T, ES_{PS2}, and ES_{PS1} (Table 3). ES_T decreased to a similar extent under both conditions indicating no apparent protection to the photosynthetic apparatus. Then the significance of increased absorption cross-section of PS1 during HI-treatment and its increased activity may be to generate the ATP molecules required for repair processes during the recovery period.

ES_{PS2} decreased apparently more during the HI-treatment in leaves preadapted to state 1 than to state 2. But this apparently greater decrease included the LHC2-decoupling induced reduction in PS2 activity, which was about 15 % of total activity (5.6/35.5, Table 2). Similarly, an about 15 % difference was found between ES_{PS2} of HI-treated leaves preadapted to state 1 and state 2 (Table 3). This explains that the "true" inhibition is about 15 % less than the apparent inhibition. Thus, the association of LHC2 with PS2 at the time HI-treatment has no additional inhibitory effect on PS2, confirming a more recent observation from *in vitro* studies (Tyystjärvi *et al* 1994).

Table 3. Effects of high-irradiance, HI (600 W m^{-2}, 30 min) treatment on ES_T, ES_{PS1}, ES_{PS2}, F_0, F_m, and F_v/F_m in sugar maple leaves preadapted to state 1 or state 2. Measurements were made immediately after treatment. Values were expressed as % of control (before HI-treatment). Means of four replicates ± SD.

	Immediately after HI-treatment in leaves preadapted to state 1	state 2
ES_T	63.7 ± 7.0	66.4 ± 9.2
ES_{PS1}	163.7 ± 29.2	59.8 ± 18.0
ES_{PS2}	53.7 ± 7.5	69.0 ± 11.4
F_0	78.8 ± 16.6	83.7 ± 11.2
F_m	33.3 ± 5.0	39.4 ± 7.1
F_v/F_m	68.1 ± 9.7	67.2 ± 6.1

We also measured modulated fluorescence in HI-treated leaves preadapted to state 1 or state 2 (Table 3). Both F_0 and F_m were higher in state 1 than in state 2. The F_v/F_m, which is an indicator of physiological state of PS2, remained similar both in state 1 (0.80 ± 0.03) and state 2 (0.78 ± 0.04) in control leaves. It decreased to a similar extent after HI-treatment in leaves preadapted to state 1 or state 2, confirming our PA observations. Thus, the percent of PS2 activity in HI-treated leaves measured as F_v/F_m (Table 3) was nearly equal to that measured as ES_{PS2} (state 2, Tables 2 and 3). Both F_0 and F_m decreased; but the decrease was marginally higher in leaves adapted to state 1 than to state 2. This also suggests the migration of LHC2 from PS2 to PS1 during HI-treatment. The decrease in F_0 may indicate the increased radiationless energy dissipation in the antenna pigments (Krause 1988, Demmig-Adams *et al.* 1989).

The non-radiative dissipation of excess radiant energy has been suggested as a protective mechanism in HI-treated leaves (Fork *et al.* 1986, Buschmann 1987, Demmig *et al.* 1988, Havaux 1989). This was indicated by measuring non-photochemical quenching of fluorescence (Krause 1988, Krause *et al.* 1988) and heat emission (Buschmann 1987, Havaux 1989). In the present study, heat emission measured in the presence of continuous background WL increased by about 12 % in HI-treated seedlings, which was smaller than the earlier reported observations on pea leaves (Havaux 1989). This increased heat emission, possibly due to increase in non-photochemical fluorescence quenching, as widely reported, may serve as a protective mechanism against excess radiation. But it is not clear from the present study whether the extent of protection offered by the increased heat emission as ES_T also diminished considerably. However, as discussed by some authors (Cleland *et al.* 1986, Krause 1988, van Wijk and van Hasselt 1993, Gilmore and Björkman 1994), a down regulation of PS2 activity, possibly due to the inactivation and without any degradation of D1 protein, may be considered as a protective mechanism. Our studies do not enable to learn the state of D1 protein of PS2 reaction center in the HI-treated leaves. The increased PS1 activity due to the increase in its absorption cross-section may be responsible for the ΔpH induced increase in thermal dissipation of the absorbed energy.

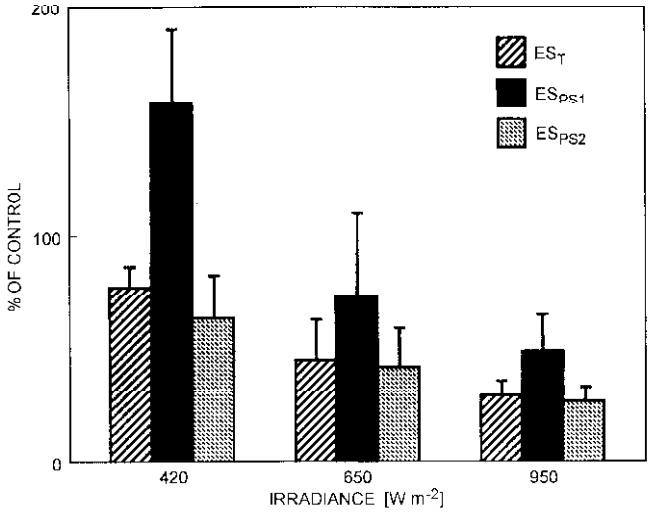

Fig. 3. Effect of different irradiances on ES_T, ES_{PS1}, and ES_{PS2} following 1 h treatment in sugar maple leaves. Conditions are as in Fig. 1*A*.

Further, in order to understand the irradiance at which the apparent increase in PS1 activity is affected, we studied the changes in ES_T, ES_{PS1}, and ES_{PS2} in leaves exposed to irradiances higher than 420 W m^{-2} for 1 h. Similar to ES_T and ES_{PS2}, ES_{PS1} also decreased at 650 W m^{-2}. A further increase of irradiance to 950 W m^{-2} caused a significant diminution of photochemical activities of both photosystems (Fig. 3). At this stage, the lack of apparent increase in ES_{PS1} can be due to the damage to PS1 population and/or due to the inactivation of protein kinase (Schuster *et al.* 1986) responsible for phosphorylation and migration of LHC2 from PS2 to PS1. The PS2 inhibition may be explained by the damage to the reaction center and an irreversible modification of D1 protein in intact leaves (Ohad *et al.* 1990, van Wijk and van Hasselt 1993). The PS1 inhibition may be due to photodestruction of iron-sulfur centers and P700 (Inoue *et al.* 1986, 1990), which differs under aerobic and anaerobic *in vitro* conditions (Inoue *et al.* 1986, Godde *et al.* 1992).

In conclusion, it can be stated that HI-treatment (420 W m^{-2}, 1 h) causes a significant increase in PS1 and a decrease in PS2 activities. The increase is due to the coupling of LHC2 to PS1 during the HI-treatment. There is a clear state 1-state 2 transition phenomenon in HI-treated leaves. The dissociation of LHC2 from PS2 during HI-treatment results in apparently (about 15 %) greater inhibition of PS2 than the true inhibition. The presence of LHC2 with PS2 (state 1) at the time of HI-treatment has no additional inhibitory effect on the PS2 activity. As ES_T decreased to a similar extent in HI-treated leaves preadapted to state 1 or state 2, it may be stated that LHC2 dissociation from PS2 or its coupling to PS1 has provided no apparent protection to the photosynthetic apparatus. At high irradiances, both PS1 and PS2 are significantly inhibited, and no increase in PS1 activity is noticed.

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