The role of inactive photosystem-II-mediated quenching in a last-ditch community defence against high light stress *in vivo*

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Photoinactivation of photosystem II (PSII), the light-induced loss of ability to evolve oxygen, is an inevitable event during normal photosynthesis, exacerbated by saturating light but counteracted by repair via new protein synthesis. The photoinactivation of PSII is dependent on the dosage of light: in the absence of repair, typically one PSII is photoinactivated per 10^7 photons, although the exact quantum yield of photoinactivation is modulated by a number of factors, and decreases as fewer active PSII targets are available. PSII complexes initially appear to be photoinactivated independently; however, when less than 30% functional PSII complexes remain, they seem to be protected by strongly dissipative PSII reaction centres in several plant species examined so far, a mechanism which we term 'inactive PSII-mediated quenching'. This mechanism appears to require a pH gradient across the photosynthetic membrane for its optimal operation. The residual fraction of functional PSII complexes may, in turn, aid in the recovery of photoinactivated PSII complexes when conditions become less severe. This mechanism may be important for the photosynthetic apparatus in extreme environments such as those experienced by overwintering evergreen plants, desert plants exposed to drought and full sunlight and shade plants in sustained sunlight.

Keywords: photoinactivation; photoinhibition; photoprotection; photosystem II; photosystem II reaction-centre quenching

1. INTRODUCTION

Photoinactivation of PSII, the loss of ability to oxidize water and evolve oxygen, is an inevitable 'wear-and-tear' consequence of normal photosynthesis. It is exacerbated when the light absorption exceeds the capacity for photosynthetic utilization. As long as a century ago, Ewart (1896) described some of the earliest observations on the photoinactivation of PSII. He assessed the capacity for oxygen evolution, and indirectly for carbon assimilation, of cells and tissues under a microscope by the bacterial method of Engelmann. Thus, he reported that 'leaves exposed, attached to the parent plant, to full sunlight till 4PM show no assimilation, though living, green and with normal chlorophyll grains'. He also observed that when left in diffuse light overnight, the leaves recovered the power of carbon assimilation, provided the prior photoinactivation was not excessive.

Plants have evolved a number of photoprotective strategies to minimize photoinactivation of PSII (for reviews see Prášil *et al.* 1992; Aro *et al.* 1993; Chow 1994, 2001; Osmond 1994; Demmig-Adams & Adams 1996; Horton *et al.* 1996; Anderson *et al.* 1997; Gilmore 1997; Melis 1999; Niyogi 1999; Öquist & Huner 2003). Interestingly, many of these photoprotective strategies depend in some way on the pH on either or both sides of the photosynthetic (thylakoid) membrane (see Chow & Hope 2002). For example, these pH-related strategies include a quenching of excitation energy that depends on a trans-thylakoid Δ pH (Horton *et al.* 1996), a de-epoxidation of violaxanthin that is promoted by such a Δ pH (Yamamoto 1979; Hager 1980; Demmig-Adams & Adams 1996; Horton *et al.* 1996; Gilmore 1997), a water-water cycle that helps to utilize excess energy while generating a Δ pH (Asada 1999), and a PSII repair cycle in which new synthesis of proteins depends on an optimal pH on the external (stromal) side of the thylakoid membrane (Bhaya & Jagendorf 1984) and on the Δ pH for the supply of adenosine triphosphate (Kuroda *et al.* 1992). Clearly, appropriate pH values on both sides of the thylakoid membrane are needed to optimize certain photoprotective strategies.

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One novel photoprotective strategy that has not yet been researched extensively was proposed more than a decade ago, namely, that a photoinactivated PSII may dissipate excitation energy efficiently as heat, thus avoiding further damage such as destruction of its antenna pigments (Krause 1988). Öquist *et al.* (1992) extended this hypothesis to include the situation where a photoinactivated PSII complex becomes such a strong sink for excitation energy that it helps to prevent neighbouring, connected PSII complexes from photoinactivation. Our recent studies with leaves have provided results that are consistent with predictions of the hypothesis, assuming that a photoinactivated PSII complex is initially only a weak sink, but later turns into a very strong sink for excitation energy when prolonged illumination has photoinactivated the majority of PSII complexes (Lee *et al.* 2001). Significantly, the residual 10–20% of PSII remains functional despite the inhibition of repair by lincomycin. Further, the operation of this photoprotective mechanism seems to require a ΔpH across the thylakoid membrane (Lee *et al.* 2002). This paper summarizes our findings relating to this last-ditch defence against high light stress, presumably a form of energy dissipation in the PSII reaction centre itself.

2. THE QUANTIFICATION OF FUNCTIONAL PSII

(a) *Determination by single turnover flashes*

Measurements of O_2 evolution per single turnover flash were used as a primary method to quantify functional PSII in leaf discs in a gas-phase oxygen electrode (Chow *et al.* 1989, 1991). This method of repetitive flash illumination was previously applied to algal suspensions (Emerson & Arnold 1932; Myers & Graham 1983). Quantification of PSII is based on the assumption that for every four single turnover, saturating light flashes, four electrons pass from two water molecules to PSII acceptors and beyond, and one oxygen molecule is evolved, per active PSII complex:

The quantification further assumes that electron acceptors in each PSII complex are in a predominantly oxidized form, on average, during repetitive flash illumination, in order to ensure that every electron transfer is successful; this is indeed the case (Chow *et al.* 1991). By comparing the gross rate of oxygen evolution with the flash frequency, the number of functional PSII complexes capable of oxygen evolution can be evaluated. The number of functional PSII complexes, so obtained, agrees on average within *ca.* 12% with the number of binding sites for the herbicide DCMU specifically associated (in a 1 : 1 stoichiometry) with PSII complexes in thylakoids isolated from many non-stressed plant species (Chow *et al.* 1989).

Figure 1 shows the percentage decrease in functional PSII in capsicum leaf discs (100% corresponding to *ca.* $1 \mu\text{mol PSII m}^{-2}$), as quantified by the oxygen yield per flash in a train of single turnover flashes, with the time of prior illumination ($1570 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) at 15°C at which repair via chloroplast protein synthesis is minimal (Lee *et al.* 2001). This figure also demonstrates the inhibition, by lincomycin, of chloroplast-encoded protein synthesis even when the temperature (25°C) and low light

Figure 1. The time-course of photoinactivation of PSII during illumination of capsicum leaf discs at $1570 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the absence of lincomycin but at a cool temperature (15°C) for up to 3.5 h. Subsequently, the leaf discs were vacuum infiltrated with a 1 mM lincomycin solution and incubated under low light and at 25°C . The presence of lincomycin inhibited chloroplast-encoded protein synthesis, and prevented recovery of PSII complexes under otherwise favourable conditions. Functional PSII was quantified by the oxygen yield per repetitive single-turnover flash. Capsicum plants were grown in low light ($100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). (Redrawn from Lee *et al.* (2001).)

($15 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were otherwise favourable for repair and recovery.

Photoinactivated PSII complexes, while unable to evolve oxygen, are still able to absorb light. Therefore, one expects the efficiency (quantum yield) of oxygen evolution to decrease with progressive photoinactivation of PSII. The quantum yield is on average $0.106 \text{ mol oxygen mol photons}^{-1}$ absorbed for a large number of non-stressed plant species photosynthesizing in limiting white light and producing a three-carbon molecule as the first stable compound (Björkman & Demmig 1987). After light stress conditions, however, the quantum yield is less than this optimal value. Figure 2 shows that the quantum yield of oxygen evolution of capsicum leaf discs decreases linearly with the decrease of functional PSII. Previously, Öquist *et al.* (1992) also reported a linear relationship for pea and *Tradescantia* leaves, albeit over a more limited range of functional PSII concentrations.

(b) *Determination by a rapid chlorophyll fluorescence method*

Oxygen measurements are inherently slow because of the need to accumulate oxygen over many flashes or over a sufficiently long period in low, continuous light. Measurements of relative chlorophyll fluorescence yield, however, are rapid, allowing quick sampling of many leaves. When such measurements are calibrated against

Figure 2. Linear correlation of the quantum yield of oxygen evolution (determined under light-limiting conditions) with the functional fraction of PSII (determined by the oxygen yield per flash). Capsicum leaf discs were illuminated under $1400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 25°C to vary the fraction of functional PSII (the fraction $1.0 \approx 1 \mu\text{mol m}^{-2}$). Some leaf discs had been vacuum infiltrated with 1 mM lincomycin to speed up photoinactivation. Capsicum plants were grown as described by Lee *et al.* (2001), except that the growth irradiance was $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The straight line is a linear regression constrained to pass through the origin.

the oxygen yield per flash, they provide a convenient and non-intrusive assay of functional PSII. Empirically, we have found that the chlorophyll fluorescence value, $(1/F_o - 1/F_m)$, where F_o and F_m correspond to the relative minimal and maximum fluorescence yield of PSII traps open and closed to incoming excitation, respectively, is a linear indicator of the functional fraction of PSII (Park *et al.* 1995a; Lee *et al.* 2001). Such a linear relationship is given in figure 3 for capsicum leaf discs pre-treated in high light to vary the concentration of functional PSII. In the calculation of $1/F_o - 1/F_m$, all fluorescence yields are relative to the F_o -value of control leaves, and the maximum $1/F_o - 1/F_m$ -value for non-stressed leaves is taken to correspond to 100% functional PSII. Thus, the functional fraction of PSII can readily be determined from chlorophyll fluorescence measurements (figure 3). This is a convenient method to use for leaves, provided an initial calibration is made of the linearity of the concentration of PSII against $1/F_o - 1/F_m$ for the plant species under investigation.

3. THE INEVITABILITY OF PHOTOINACTIVATION OF PSII

It is commonly said that light-induced loss of PSII activity occurs when absorption of light exceeds the capacity of chloroplasts to utilize the excitation energy.

Figure 3. Linear correlation of the chlorophyll fluorescence value $1/F_o - 1/F_m$ with the proportion of functional PSII (measured by the oxygen yield per flash). Capsicum leaves were taken from plants grown under 500 (high light) or $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (low light). Leaf discs were illuminated under varied irradiance, either in air with 1% CO_2 or floated on water in normal air, in the presence or absence of lincomycin. Open circles, high light grown, 1% CO_2 ; open diamonds, low light grown, 1% CO_2 ; filled circles, high light grown, floated; filled diamonds, low light grown, floated. (Redrawn from Lee *et al.* (2001).)

Accordingly, there is a mistaken belief that no photoinactivation of PSII occurs in the low light experienced by many leaves in a canopy. However, gross photoinactivation of PSII occurs even in low light; it is only the continual repair of PSII *in vivo* that ensures little net loss of function in low light.

(a) Photoinactivation of PSII depends on the light dose

Combinations of irradiance and duration of illumination that give the same total incident photons per square metre, 'photon exposure' (Bell & Rose 1981), will produce the same extent of photoinactivation of PSII, other things being equal. This is the law of reciprocity, found to hold in a number of photosynthetic organisms subjected to light stress (Jones & Kok 1966; Nagy *et al.* 1995; Park *et al.* 1995a, 1996; Lee *et al.* 1999). Figure 4 shows an example from leaf discs of high light grown capsicum plants that were illuminated in the presence of lincomycin (an inhibitor of protein synthesis in the chloroplast and, therefore, of repair after photoinactivation) while being floated on water in normal air. The law of reciprocity clearly holds: illumination at three irradiances, ranging from the growth irradiance to that of almost full sunlight, gave the same curve for the decline of functional PSII with photon exposure.

Possibly, the basis for the reciprocity of irradiance and the duration of illumination lies in the exciton-radical pair equilibrium in PSII. In this equilibrium model, a photon visits a PSII reaction centre and causes primary charge

Figure 4. The decrease in the functional fraction of PSII, determined from $(1/F_o - 1/F_m)$, with increase in photon exposure. The photon exposure was varied by varying the illumination time at one of three irradiances, 460 (open circles), 900 (filled circles) and 1800 (open inverted triangles) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Leaf discs, taken from capsicum plants grown under $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (high light), were illuminated in the presence of lincomycin. Up to a photon exposure of *ca.* 10 mol m^{-2} , the decrease could be described by a single exponential, with a slope of $-1.46 \times 10^{-7} \text{ mol PSII (mol photons)}^{-1}$ at very low photon exposures, as indicated by the dashed line. (Redrawn from Lee *et al.* (2001).)

separation (electron transfer from the special chlorophyll P_{680} to pheophytin Ph). A charge recombination between P_{680}^+ and Ph^- then occurs, yielding an exciton in the antenna. The exciton may again induce another charge separation. In this way, an exciton may visit a reaction centre several times before being finally trapped photochemically or dissipated as heat (Schatz *et al.* 1988). It is possible that almost every absorbed photon, including those that are finally dissipated in the antenna as heat, may have made a number of visits to a reaction centre, each time forming a radical pair. That is, every absorbed photon contributes to the formation of P_{680}^+ and, therefore, to possible photoinactivation of PSII by this extremely oxidizing cation (Barber 1995; Anderson *et al.* 1998). This may be a basis for the dependence of photoinactivation on photon exposure, and for the reciprocity law as mentioned above.

The validity of the law of reciprocity depends on an effect determined by the number of photons absorbed, rather than the rate at which they arrive (irradiance). Reciprocity of irradiance and duration of illumination may not hold if, for example, the absence of lincomycin allows chloroplast protein synthesis to occur at a rate that varies with irradiance (Chow 2001), or if the rate of photosynthetic utilization of absorbed light varies with time in the induction phase during illumination following a dark period (Shen *et al.* 1996).

Table 1. The maximum quantum yields of photoinactivation of PSII (in $\mu\text{mol PSII per mol photons}$) in capsicum leaf discs (in air enriched with 1% CO_2).

	low light grown	high light grown
– lincomycin	0.052	0.035
+ lincomycin	0.12	0.10

(b) *The quantum yield of photoinactivation of PSII*

In capsicum leaf discs, over the range of photon exposures up to *ca.* 10 mol m^{-2} , the decline in functional PSII was mono-exponential (figure 4), much like a curve of radioactive decay with time, where atomic nuclei decay independently. This is also true for pumpkin (Tyystjärvi *et al.* 1994) and *Tradescantia* (Park *et al.* 1996). The steepest decline of functional PSII complexes occurs at low photon exposures. The magnitude of the steepest slope is the maximum quantum yield of photoinactivation of PSII. The maximum quantum yields of photoinactivation of PSII (in $\mu\text{mol PSII mol}^{-1} \text{ photons}$) in capsicum leaf discs (in air enriched with 1% CO_2) are estimated to be as in table 1 (Lee *et al.* 1999).

By comparison, the maximum quantum yield of photoinactivation of PSII in capsicum leaf discs floated on water in normal air is estimated from the decay coefficient in figure 4 ($0.146 \text{ m}^2 (\text{mol photons})^{-1}$) as $0.146 \text{ m}^2 (\text{mol photons})^{-1} \times 1.0 \mu\text{mol PSII m}^{-2} \approx 0.15 \mu\text{mol PSII (mol photons)}^{-1}$, because the fully functional PSII population is close to $1 \mu\text{mol PSII m}^{-2}$ (Lee *et al.* 1999). This value in normal air is slightly higher than that obtained in the presence of 1% CO_2 , almost certainly due to a limitation of photosynthesis by gas diffusion when leaf discs are floated on water. At higher photon exposures, the quantum yield of photoinactivation, given by the slope at a point on the curve, declines because fewer PSII complexes are available for photoinactivation: non-functional PSII complexes absorb light but contribute no new photoinactivated complexes (Park *et al.* 1995b). A quantum yield of $0.1 \mu\text{mol PSII mol photons}^{-1}$ (in the absence of repair) means that, for every 10^7 incident photons, one member among a collection of PSII complexes is inactivated. Note that this does not necessarily mean that *each* PSII complex is inactivated after it has processed 10^7 photons.

At a quantum yield of photoinactivation of $0.1 \mu\text{mol PSII mol photons}^{-1}$ in the absence of repair, one can calculate the rate of gross inactivation that must be occurring in any light environment. At 25% full sun ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and assuming a typical initial content of $1 \mu\text{mol functional PSII m}^{-2}$, the maximum gross rate of photoinactivation is $500 \times 10^{-6} \text{ mol photons m}^{-2} \text{s}^{-1} \times 10^{-7} \text{ mol PSII (mol photons)}^{-1} \times 3600 \text{ s h}^{-1} / 10^{-6} \text{ mol PSII m}^{-2} = 18\% \text{ h}^{-1}$. This rate of photoinactivation decreases as the functional fraction of PSII decreases. Nevertheless, on one or two sunny days, the entire population of PSII complexes may have to be repaired by *de novo* synthesis and incorporation of a new D1 protein into PSII.

(c) *The probability of photoinactivation of PSII*

To a certain extent, the photoinactivation of PSII complexes can be considered to be analogous to the decay of

radioactive atoms. In the latter, identical atoms decay independently, with a probability that is directly proportional to an increment in time, resulting in an exponential decline in the population of radioactive atoms. Similarly, if it is assumed that (i) PSII complexes in a homogeneous population are photoinactivated independently, at least in the early to middle stages of the population decline (see § 4a), and (ii) the probability p of photoinactivation is directly proportional to an increment in photon exposure Δx , i.e. $p = k\Delta x$, where k is a constant, then one can deduce that the number of functional PSII complexes, y , decreases exponentially with photon exposure from an initial value y_0 (Lee *et al.* 1999): $y = y_0 \exp(-kx)$. The quantum yield of photoinactivation at a given value of y is the slope at that point, and should not be confused with k , the probability per unit photon exposure. Further, since light treatments are usually carried out for different durations, t , at a fixed irradiance, I , y is usually plotted against t . Then the rate coefficient for the exponential decay is kI : $y = y_0 \exp[-(kI)t]$. Obviously, the rate coefficient is directly proportional to the irradiance, as observed (Tyystjärvi & Aro 1996; Lee *et al.* 2001).

4. SEVERELY PHOTOINACTIVATED PSII COMPLEXES PROTECT THEIR FUNCTIONAL NEIGHBOURS

(a) *Residual functional PSII complexes survive prolonged illumination despite the presence of lincomycin*

Krause (1988) hypothesized that a photoinactivated PSII complex may dissipate excitation energy efficiently as heat, thereby preventing further damage to itself, e.g. destruction of antenna pigments. Öquist *et al.* (1992) took this hypothesis further by proposing that a photoinactivated PSII complex, by acting as an energy sink, may protect neighbouring, connected functional PSII complexes from photoinactivation. Initial attempts to test this hypothesis (Tyystjärvi *et al.* 1994; Lee *et al.* 1999) did not uncover evidence consistent with predictions of the hypothesis. Later, however, Lee *et al.* (2001) extended the light treatment of capsicum leaf discs to large photon exposures which decreased the functional fraction of PSII to 30% or less, and, surprisingly, observed a resilient population which survived large photon exposures despite the inhibition of D1 protein synthesis by lincomycin. The residual sub-population of PSII is depicted in the semi-log plot in figure 5a for leaf discs of low-light grown capsicum illuminated in the presence of lincomycin. It is seen that the decline in functional PSII is mono-exponential until *ca.* 30% of functional PSII remains; then deviation from a single exponential decline occurs abruptly, leaving a relatively constant fraction of functional PSII. A similar observation was also made in high light grown capsicum (figure 5b), high light grown pea (Park *et al.* 1997) and low light grown wild-type *Arabidopsis* (W. S. Chow and B. J. Pogson, unpublished data). We propose that this mode of photoprotection, which comes into operation after the majority of PSII complexes have been photoinactivated, be named IPQ. A scheme of this mode of photoprotection is depicted in figure 6: light energy absorbed by an active PSII complex is efficiently transferred to pho-

Figure 5. The decrease in the functional fraction of PSII with photon exposure given to leaf discs of capsicum plants grown under (a) low light ($100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) or (b) high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Leaf discs were illuminated at 460 (open circles), 900 (filled circles) or 1800 (open triangles) $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the presence of lincomycin. Data are replotted from Lee *et al.* (2001). The functional fraction of PSII was determined from $1/F_0 - 1/F_m$, and plotted on a semi-log scale to show the exponential decrease up to a photon exposure of about 10 mol m^{-2} . Note the stable fraction (*ca.* 0.2) of functional PSII even at high photon exposures and in the presence of lincomycin.

toinactivated neighbours which act as strong sinks to readily dissipate the excitation energy as heat. In this way, the residual active PSII complexes can be protected against photoinactivation even if no repair by D1 protein synthesis is allowed to occur.

(b) *A simple kinetic model incorporating IPQ*

The decrease in functional PSII in figure 5 was mono-exponential until *ca.* 30% remained. This indicates that PSII complexes were photoinactivated independently during the exponential phase. This could be due to inactive PSII complexes being only weak sinks initially. Later, when less than 30% of functional PSII complexes remained, a strongly protective mechanism seemed to

Figure 6. A schematic diagram showing that excitation energy absorbed into an active PSII is shunted to connected photoinactivated neighbours. Provided the photoinactivated neighbours are strong sinks for excitation energy, some active PSII complexes may be protected against photoinactivation. We term this mechanism IPQ.

operate. Lee *et al.* (2001) proposed that the small residual sub-population of functional PSII is protected by photoinactivated neighbours, which presumably have been converted from weak to strong sinks. At time t , the rate of photoinactivation is $k_i f$, where f is the fraction of functional PSII and k_i the 'intrinsic' rate coefficient. The decrease in functional PSII, in lincomycin-treated capsicum leaf discs with illumination time, could be modelled by postulating that k_i is significantly modified by a factor $1/[\varepsilon(1-f)^{10} + 1]$ when more than *ca.* 70% of the PSII complexes have been photoinactivated; at a lesser extent of photoinactivation, the factor has a negligible effect. The factor $1/[\varepsilon(1-f)^{10} + 1]$ is a purely phenomenological description of an effect that comes into operation only after a heavy loss of functional PSII complexes. The rate of photoinactivation then becomes $df/dt = -k_i f / [\varepsilon(1-f)^{10} + 1]$. Solving this differential equation gives t as a function of f (Lee *et al.* 2001), which was fitted to the experimental data for an appropriate value of ε (chosen by trial and error to give a good fit) and k_i . The curve in figure 7a was obtained with $\varepsilon = 50$ and $k_i = 0.75 \text{ h}^{-1}$ for leaf discs of low light grown capsicum treated with lincomycin to inhibit repair. At a given functional fraction of PSII, the effectiveness with which photoinactivated PSII complexes protect their functional neighbours is indicated by the value of ε : the larger the value, the more effective is the mechanism. The manner in which the value of ε varies with experimental conditions gives an indication of what is required to operate this photoprotective mechanism.

Figure 7. Time-course of the decrease in the functional fraction of PSII (determined from $1/F_0 - 1/F_m$) in leaf discs illuminated at $1800 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the presence of lincomycin. Leaf discs were taken from capsicum grown under low light ($100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), and illuminated in (a) the absence ($\varepsilon = 50$, $k_i = 0.75 \text{ h}^{-1}$) or (b) presence ($\varepsilon = 5$, $k_i = 1.72 \text{ h}^{-1}$) of nigericin. The curves are fitted according to a simple kinetic model. Data in (a) are replotted from Lee *et al.* (2001), and those in (b) are replotted from Lee *et al.* (2002).

(c) Apparent requirement for a ΔpH to operate the IPQ photoprotective mechanism

Figure 7b shows the fitting of a curve to the data with values of $\varepsilon = 5$ and $k_i = 1.72 \text{ h}^{-1}$ for leaf discs of low light grown capsicum in the presence of nigericin (and lincomycin as for figure 7a). Nigericin is a molecule that exchanges protons out of the internal (lumen) side of thylakoids for an influx of K^+ ions. When nigericin is present, it is seen that ε is much smaller. As nigericin is an uncoupler that diminishes the ΔpH across the thylakoid membrane, it appears that a high value of ε requires a large ΔpH . That is, the effectiveness with which photoinactivated PSII complexes protect their functional neighbours seems to depend on a ΔpH (Lee *et al.* 2002).

Table 2 gives some estimates of values of ε under various treatment and growth conditions. It is seen that regardless of the growth irradiance, nigericin decreased ε by at least an order of magnitude. Interestingly, the value of ε in the presence of nigericin was higher in leaves taken from plants grown in low light (5) as compared with high

Table 2. Summary of values of ε .

(The value ε is a measure of the effectiveness with which photoinactivated PSII complexes protect their functional neighbours, estimated as described by Lee *et al.* (2001). When used, [nigericin] = 5 μ M. Values with an asterisk are from Lee *et al.* (2001), while values obtained in the presence of nigericin are from Lee *et al.* (2002).)

growth irradiance (μ mol photons $\text{m}^{-2} \text{s}^{-1}$)	treatment irradiance (μ mol photons $\text{m}^{-2} \text{s}^{-1}$)	chemical treatment	ε
low			
100	1800	+ H ₂ O	50*
100	1800	+ nigericin	5
high			
500	1800	+ H ₂ O	50*
500	1800	+ nigericin	0.5

light (0.5). It is well known that low light grown plants have chloroplasts with large antennae associated with the two photosystems. In particular, the large antenna of PSII, as reflected in the enrichment of chlorophyll *b* and enlarged grana structures, probably results in better connectivity among PSII complexes, and in greater photoprotection when energy-dissipating PSII complexes can help to avoid over-excitation of their neighbours (Anderson & Aro 1994).

When thylakoids are isolated from pea leaves and subjected to photoinhibitory light treatment in the presence of nigericin, the decline in functional PSII is strictly mono-exponential (Vavilin *et al.* 1998). The mono-exponential decrease in functional PSII means that for every active PSII the probability of photoinactivation in unit time (at a given irradiance) is constant throughout the light treatment and independent of the concentration of photoinactivated PSII units (Vavilin *et al.* 1998; Lee *et al.* 1999). That is, each PSII complex appears to be inhibited independently with no influence of connectivity between PSII complexes. This is expected to be the case according to our present kinetic model if $\varepsilon \approx 0$ due to nigericin dissipating the Δ pH across the thylakoid membrane, i.e. photoprotection of functional PSII by photoinactivated neighbours requires a Δ pH to operate.

It is not clear how an acidic lumen or a trans-thylakoid Δ pH can regulate the IPQ photoprotective mechanism. However, protonation of proteins can certainly have profound effects on, for example, energy-dependent quenching (Horton *et al.* 1996; Gilmore 1997) and calcium binding to PSII (Krieger & Weis 1993). Horton *et al.* (1996) have described four targets of protonation, each capable of regulating energy flow in PSII. Further, it has been shown by high-resolution electron microscopy that either illumination or addition of H⁺ in the dark leads to a contraction of thylakoid membranes and a decrease in the spacing between them (Murakami & Packer 1970). In this context, it is interesting to note that grana formation allows a controlled dissipation of excitation energy (Horton 1999): at low light, the presence of grana prevents a collapse of the dense array of proteins into a highly dissipative state; in high light, protonation of protein residues is prevalent, favouring closer packing and enhancing non-photochemical quenching. Given these various pro-

found effects of protonation on the PSII core and its peripheral chlorophyll *a/b* complexes, one expects that an acidic luminal pH may play a central part in the IPQ photoprotective mechanism. For example, an acidic luminal pH may render severely photoinactivated PSII complexes strongly quenching, while also promoting the much closer connectivity between an active PSII complex and a few inactive neighbours. Then, excitation energy absorbed into functional PSII complexes will be readily transferred to connected, non-functional PSII neighbours, which serve as very strong sinks (figure 6).

(d) Energy dissipation in photoinactivated PSII reaction centres

How does a severely photoinactivated PSII complex act as a strong sink for excitation energy? When the excitation energy reaches a PSII reaction centre, a temporary charge separation occurs, leaving an electron on the acceptor side and an electron hole on the donor side of PSII. Because a photoinactivated reaction centre is incapable of transferring an electron further down the chain, a charge recombination possibly takes place directly to form the ground state rather than recreating an excited state of chlorophyll. In this way, the light energy could be dissipated as heat. Charge recombination directly to the ground state has been proposed for the slowly reversible non-photochemical quenching of chlorophyll fluorescence in moderately photoinactivated pea thylakoids (Richter *et al.* 1999). It could conceivably occur to a much greater extent in severely photoinactivated PSII reaction centres that have been subjected to extensive modification, for example, by oxygenation (Miyao 1994; Barber 1998).

A specific mechanism has been proposed for reaction-centre dissipation in over-wintering pine needles. On the acceptor side of PSII are two quinone acceptors, Q_A and Q_B. The redox potential of the Q_B⁻/Q_B couple is normally less negative than that of Q_A⁻/Q_A, allowing further stabilization of charge separation. However, from thermoluminescence measurements of over-wintering pine needles, Ivanov *et al.* (2002) concluded that severe photoinactivation of PSII renders the redox potential of the Q_B⁻/Q_B couple closer to that of Q_A⁻/Q_A. This presumably favours the distribution of electrons on Q_A, increasing the population of Q_A⁻. In turn, the increased [Q_A⁻] increases the probability of non-radiative charge recombination between Q_A⁻ and an electron hole on the donor side of PSII, including the special chlorophyll cation, P₆₈₀⁺ (Krieger-Liszkay & Rutherford 1998; Manuel *et al.* 1999; Vavilin & Vermaas 2000). Such a charge recombination, if resulting in a ground state directly and not an excited state, could allow the reaction centre to act as an energy sink.

However, to enhance charge recombination of Q_A⁻ and P₆₈₀⁺ to the ground state directly would probably require closer proximity of the redox potentials of the couples Q_A⁻/Q_A and P₆₈₀⁺/P₆₈₀. A smaller redox potential difference between these two couples will enhance radiationless transition in a way that is analogous to an increased probability of radiationless transition at a smaller energy gap in large molecules (Englman & Jortner 1970). Enhanced radiationless transition in turn offers safe dissipation of excitation energy. A smaller redox potential difference could be achieved in two ways, each with an additional photo-

protective effect. First, the redox potential of Q_A/Q_A^- could be rendered less negative, as occurs when DCMU binds to PSII (Krieger-Laszak & Rutherford 1998). Then the redox potential difference between Q_A/Q_A^- and Ph/Ph^- is increased, impairing a back electron transfer and inhibiting the formation of the P_{680}^+/Ph^- state. In this way, the formation of triplet P_{680} is minimized, thereby avoiding the formation of highly reactive singlet oxygen. Second, the redox potential of P_{680}/P_{680}^+ could be rendered less positive, as proposed by Anderson & Chow (2002). The less oxidizing P_{680}^+ implies less likelihood of further damage induced by light. In order to test these ideas, there is an urgent need to determine the electrochemical potentials of the redox cofactors in PSII following photoinactivation.

(e) *Relevance of IPQ photoprotection to extreme physiological stress conditions*

The IPQ photoprotective mechanism may be very important in plants experiencing severe multiple stress conditions. One example is over-wintering evergreen plants. They are exposed to freezing temperatures which limit other photoprotective strategies, including the utilization of absorbed light energy by photosynthesis; at the same time, their leaves must survive a combination of freezing temperatures and high light if they are to realize leaf longevity as an advantage in climatic zones with a short growing season and in regions with nutritionally poor soil (Öquist & Huner 2003). Other examples include water-stressed desert plants in full sunlight, extreme shade plants suddenly exposed to sustained sunlight, and so on. Possibly, the retention of a fraction of functional PSII complexes, by initiating linear electron flow and stimulating photosynthesis, aids recovery of the photoinactivated complexes on returning to favourable conditions such as warmer temperatures and lower light. If so, the PSII complexes function as a cooperative community: *in extremis*, photoinhibited complexes appear to protect their functional neighbours, which later help their photoinactivated counterparts to recover.

5. CONCLUDING REMARKS

The photoinactivation of PSII complexes is an inevitable event in oxygenic photosynthesis that uses water as a substrate. The photoinactivation of PSII depends on the photon exposure, and therefore, occurs even in weak light given over a longer time. The exact dependence of PSII photoinactivation on photon exposure, however, is modulated by mitigating factors. Under a given set of conditions, one sometimes observes reciprocity of irradiance and duration of illumination, particularly when D1 protein synthesis is inhibited. To minimize photoinactivation of PSII, plants have evolved numerous photoprotective strategies. An interesting, possibly 'last-ditch', strategy is the photoprotection of functional PSII complexes by photoinactivated neighbours when the residual functional PSII complexes are sustained even in the absence of D1 protein synthesis. This IPQ appears to operate only when the majority (70–80%) of PSII complexes have been photoinactivated, and when a ΔpH is present. The residual population of functional complexes, by providing a ΔpH , in turn may play a crucial part in the recovery of photoinacti-

vated PSII units when favourable conditions return: a kind of mutual aid and cooperation.

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Discussion

B. Andersson (Rector, Linköping University, Linköping, Sweden). I have a question and a comment. I agree that it is hard to photoinactivate all PSII centres; even *in vitro*, one often has 10–20% active centres remaining. Do you think it is just a statistical phenomenon that 20% of PSII remains and is being protected by its inactivated neighbours or are these surviving PSII centres different in some respect, making them more resistant? Personally, I believe that the surviving centres are the so-called PSII β centres that are very hard to photoinactivate *in vitro*.

I am surprised that the repair of photodamaged PSII is so efficient at 35 °C, because at this temperature the manganese cluster starts to be destabilized and there is structural rearrangement in the PSII antenna.

W. S. Chow. It is interesting that even *in vitro* some PSII centres are hard to photoinactivate, even though the sample is in a homogeneous suspension. This tends to rule out the possibility that the residual PSII centres in leaves survive not because they are shaded from the light.

As to whether these surviving PSII centres are PSII β units, the smaller light-harvesting antenna of PSII β centres would tend to mitigate against photoinactivation. However, PSII in the chlorophyll *b*-less mutant of barley is more easily photoinactivated, despite the smaller antenna (Leverenz *et al.* 1992), as is also the case in a chlorophyll *b*-less of rice (W.S. Chow and I. Terashima, unpublished data). Certainly, if there is an effective quenching mechanism, much of the absorbed energy can be dissipated; the antenna is effectively small, and the residual PSII centres will resemble PSII β centres.

Our analysis (Lee *et al.* 2001) separates the rate coefficient of repair from that of photoinactivation. The rate coefficient of photoinactivation at 35 °C, indeed, is about twice as large as that at 15 °C, presumably due to a destabilized PSII structure, as you pointed out.

I. Vass (*Department of Biology, Biological Research Centre, Szeged, Hungary*). What is the reason for the break in the inactivation curve? Continuous production of inactive PSII centres that protect the others would result in a continuous inactivation curve with no distant break. Did you consider the effect of the light gradient through the leaf and the fact that the lower layers would be protected from high light?

W. S. Chow. A light gradient, allowing PSII units in the lower layers to be shaded from the light, does not seem to explain the existence of a residual population, as this residual population was very much decreased when high CO₂ or nigericin was present during the photoinhibition treatment (Lee *et al.* 2002). We think that inactive PSII centres are only weakly quenching initially, so that there is no break from the straight line in the semi-log plot until much stronger quenching centres are formed much later.

S. Santabarbara (*Department of Biology, Queen Mary*

College, University of London, London, UK). We have been measuring the relationship between the excited state population in PSII antenna and photoinactivation and have shown that the quenching can induce a protection that is, at maximum, about 30% of what is expected on the basis of the reciprocity rule. Do you think that inactive PSII units protect active PSII, in your hypothesis, by a quenching process, because our data seem to indicate that this is rather unlikely.

We think that, at room temperature, the quenching will be nearly homogeneous over all PSII complexes, because energy transfer is much faster than the excited state decay processes.

W. S. Chow. The rate coefficient for photoinactivation (when no repair is allowed to occur) increases linearly with irradiance (Tyystjärvi & Aro 1996; Lee *et al.* 2001). If strongly quenching PSII centres were formed that are excitonically connected to functional PSII neighbours, there is effectively less light available for photoinactivation, and one would expect photoprotection to occur. Your finding that the quenching of singlet excited states in the PSII antenna by quenchers such as *m*-dinitrobenzene offers little photoprotection (Santabarbara *et al.* 2001) is certainly interesting, and deserves further study.

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GLOSSARY

- DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethyl urea
IPQ: inactive PSII-mediated quenching
PSII: photosystem II