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The Photoinactivation of Photosystem II in Leaves: A Personal Perspective

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a, a parameter that describes how effectively photoinactivated PS II units protect their functional neighbours; Car, carotenoids; ΔpH, transthylakoid pH difference; D1 protein, *psbA* gene product in the PS II reaction centre; *f*, functional fraction of PS II; F_v/F_m, the ratio of variable to maximum chlorophyll *a* fluorescence; k_d, rate coefficient for degradation of D1 protein; k_i and k_r, rate coefficient for photoinactivation and repair of PS II, respectively; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; P680, the primary electron donor in the PS II reaction centre; Ph, pheophytin; PS, photosystem; Q_A, first quinone acceptor of an electron in PS II; R_s, the gross rate of D1 protein synthesis.

Key words: photoinactivation, photosystem II

INTRODUCTION

A paradox of photosynthesis is that light is both required for the process and detrimental to the photosynthetic apparatus. In C_3 photosynthesis under light-limiting conditions, about 9 to 10 photons are sufficient for the evolution of one O_2 molecule [15, 23]. This "quantum requirement" of 9 to 10 photons per O_2 molecule is consistent with half of the photons driving (in a stoichiometry of one electron per photon, with some loss) each of two photosystems working in series in the reaction: $2H_2O \rightarrow O_2 + 4H^+ 4e^-$. The electrons released from water are transferred along a chain consisting of:

Photosystem II components → plastoquinone pool → cytochrome *b/f* complex → plastocyanin → photosystem I components → $NADP^+$

Photosystem (PS) II is the pigment-protein complex responsible for the photooxidation of water molecules. It has to generate oxidants that are sufficiently strong to oxidise water. Because of its role in forming such strong oxidants, PS II is highly susceptible to damage by the strong oxidants themselves, particularly in strong light. For example, a leaf exposed to full sun of 2000 μmol photons m^{-2} s^{-1} absorbs about 1700 μmol photons m^{-2} s^{-1}. If the light-saturated rate of photosynthesis corresponds to the evolution of 40 μmol O_2 m^{-2} s^{-1}, and the evolution of each O_2 molecule requires 10 photons, then 400 μmol photons m^{-2} s^{-1} are sufficient to sustain the maximum photosynthetic rate. The rest of the photons need to be dissipated harmlessly as heat, with a small proportion (~1%) as chlorophyll fluorescence. Understandably, the photosynthetic apparatus has evolved a number of photoprotective strategies to cope with excess light [17, 22, 32, 49, 56]. Even so, there is a small but significant probability that PS II complexes are inactivated by light, and consequently are unable to evolve oxygen.

Once photoinactivated, PS II needs to be repaired by *de novo* synthesis of the D1 protein to replace the damaged D1 protein in the PS II reaction centre [5, 45, 67]. If repair of the photodamage occurs at a sufficient rate, little net loss of function will occur. But if repair is retarded or photoinactivation is accelerated by another environmental stress such as chilling, dysfunction of PS II results. Any net loss of PS II function will decrease photosynthetic efficiency measured in low light [65], and when more than about 40% of PS II complexes are inactivated, will also limit the maximum photosynthetic rate in high light [29, 41, 53].

This paper represents a view of the photoinactivation of PS II that is partly shaped by research done in my collaboration with others, especially my Korean collaborators. Far from being an exhaustive review, it focuses on certain aspects of photoinactivation of PS II. It examines the quantum yield of photoinactivation of PS II, and surveys the possible reactive chemical species responsible for the photoinactivation of PS II. It argues that the strongest oxidant in photosynthesis, $P680^+$, may be primarily responsible for the photoinactivation of PS II. Further, it examines semi-quantitatively two of the photoprotective strategies that mitigates against photoinactivation of PS II: (1) recovery from photoinactivation via D1 protein synthesis and (2) the mechanism whereby photoinactivated PS II complexes may under

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certain conditions protect functional neighbours from photoinactivation.

The inevitability of photoinactivation of PS II

It is commonly said that light-induced loss of PS II activity occurs when the absorbed light exceeds the capacity of chloroplasts to utilize the excitation energy. This view, however, conceals the fact that gross photoinactivation of PS II occurs even in low light, and that it is the continual repair of PS II *in vivo* that ensures little *net* loss of function in low light.

Photoinactivation of PS II depends on the light dose

Combinations of irradiance and duration of illumination that give the same total incident photons m^{-2} ('photon exposure') [12] will produce the same extent of photoinactivation of PS II, *other things being equal*. This is the law of reciprocity, found to hold in a number of photosynthetic organisms subjected to light stress [33, 41, 48, 59, 61]. In a plot of the decline in functional PS II complexes m^{-2} against photon exposure (photons m^{-2}) for pea leaf discs in an atmosphere enriched with 1.1 % CO_2 [3], for example, varying the irradiance at constant duration of illumination gives the same curve as varying the duration at constant irradiance (Fig. 1). Thus, the law of reciprocity holds in the case of leaf discs treated with water only (upper curve), and it also holds for leaf discs treated with lincomycin, an inhibitor of the synthesis of chloroplast-encoded proteins, particularly D1 protein (lower curve). However, different curves are obtained for the absence or presence of lincomycin. This is because inhibition of repair by lincomycin increases the net photoinactivation of PS II

In leaf discs of high-light grown capsicum that are illuminated in the presence of lincomycin while being floated on water in normal air, reciprocity also clearly holds. Figure 2A shows a semi-logarithmic plot of the decline in the functional PS II content with photon exposure when leaf discs are illuminated at 460, 900 or 1800 μmol m^{-2} s^{-1} for various durations. The points essentially fall on the same curve, regardless of the irradiance. However, reciprocity does not hold when capsicum leaf discs are illuminated in *normal* air in the *absence* of lincomycin (Fig. 2B). This result contrasts with observations with pea leaf discs illuminated in CO_2-enriched air in the absence of lincomycin (Fig. 1, upper curve).

It is not certain what causes this discrepancy, other than that different plant species are being compared. Perhaps, enrichment of air with 1% CO_2, just like the presence of lincomycin, has a bearing on reciprocity. Specifically, enrichment of air with CO_2 may to some extent inhibit D1 protein synthesis, as does lincomycin. If so, the law of reciprocity is more likely to apply obeyed when D1 protein synthesis is inhibited.

What is the evidence for inhibition of D1 protein synthesis by high CO_2? Lee *et al.* (2001) determined the rate coefficients of repair (k_r) and photoinactivation (k_i), processes which occur simultaneously. Lee, Hong and Chow (submitted) used this method to determine k_r (and k_i) in capsicum leaf discs illuminated in air that was enriched with >1% CO_2, and found the values of k_r shown in Table 1, as compared with values obtained in normal air. It is seen that k_r was close to zero for the irradiances 460 and 1800 μmol m^{-2} s^{-1}. At these

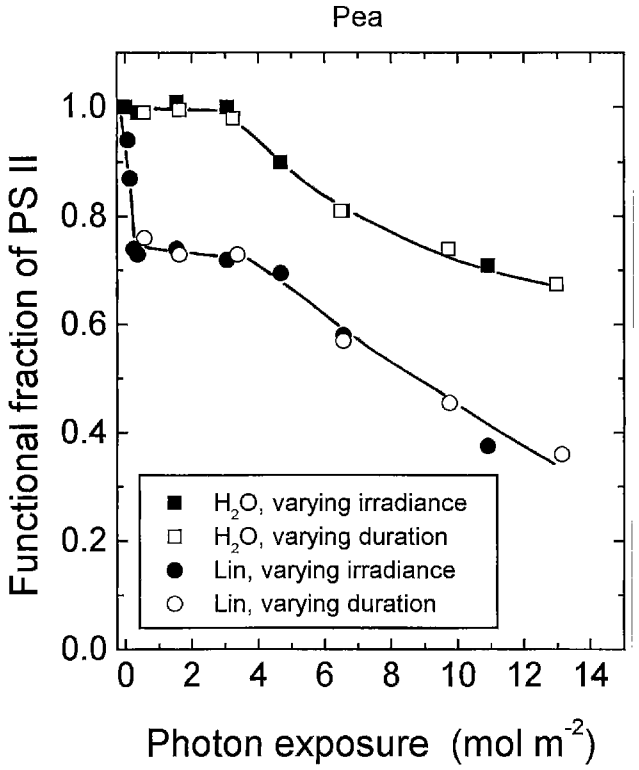


Fig. 1. Decline in the number of functional PS II complexes as a function of photon exposure (light dose) in leaves of pea plants grown in moderate light (250 μmol m^{-2} s^{-1}). The initial value of 100% corresponds to 1.06 μmol PS II m^{-2}. Leaves were either treated with water (H_2O) or lincomycin (Lin), an inhibitor of chloroplast-encoded protein synthesis. Leaf discs were illuminated individually in a chamber through which humidified air enriched with 1.1% CO_2 was passed. The leaf discs were illuminated at either varying irradiance for a fixed duration or varying duration at a fixed irradiance. Replotted from Anderson *et al.* (1997).

Table 1. The rate coefficient for repair k_r (h^{-1}), determined for capsicum illuminated at varied irradiance and in normal air or air enriched with more than 1% CO_2. Results of for normal air are taken from Lee. *et al.* 2001, and those for >1% CO_2 are from H.-Y. Lee, Y.-N. Hong and W. S. Chow (submitted).

Irradiance (μmol photons m^{-2} s^{-1})	460	900	1800
	Rate coefficient for repair, k_r (h^{-1})		
Normal air	2.40	1.37	1.25
Air with >1% CO_2	~ 0	0.043	0.019

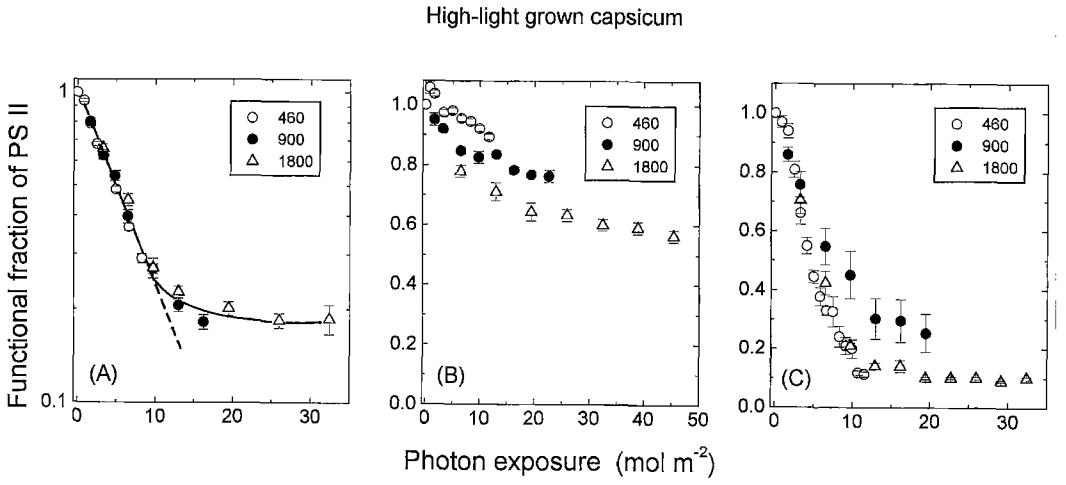


Fig. 2. The decrease in the content of functional PS II with photon exposure given to leaf discs of capsicum plants (grown under high light, 500 μmol m^{-2} s^{-1}) in the presence of lincomycin (A), absence of lincomycin (B) or in the presence of high CO_2 (> 1%). The irradiance was 460, 900 or 1800 μmol m^{-2} s^{-1}. Panels (A) and (B) are re-calculated from Lee *et al.* (2001), while Panel (C) contains unpublished data of Lee, Hong and Chow.

two irradiances, reciprocity holds, as shown in Fig. 2C. However, the data points for 900 μmol m^{-2} s^{-1} lie on a different curve, even though k_r was also quite small (only slightly larger). Understanding when reciprocity holds and when it does not hold will require further experimentation.

It has been reported that reciprocity does not hold for the photoinactivation of PS II by cumulative exposure to light pulses (up to 100 s) during the induction period of photosynthesis [73]. They found that shorter pulses are more effective at photoinactivation of PS II than longer pulses given at the same cumulative photon exposure. This is understandable since the rate of photosynthetic during photosynthetic induction, and hence the ability to utilize absorbed photons, increases in a time-dependent manner, so that the duration of illumination does matter.

The quantum yield of photoinactivation of PS II

In capsicum leaf discs, as is also true for pumpkin [80] and Tradescantia [62], PS II is photoinactivated with an approximately single-exponential dependence on photon exposure [41, 42]. The steepest decline of functional PS II complexes occurs at low photon exposures. The magnitude of the steepest slope has units μmol PS II per mol photons, and is the maximum quantum yield of photoinactivation of PS II. The maximum quantum yields of photoinactivation of PS II (in μmol PS II per mol photons) in capsicum leaf discs (in air enriched with 1% CO_2) are estimated to be [41]:

	Low-light grown	High-light grown
- lincomycin	0.052	0.035
+ lincomycin	0.12	0.10

At higher photon exposures, the quantum yield of photoinactivation declines because non-functional PS II complexes absorb light but contribute no new photoinactivated complexes. A quantum yield of 0.1 μmol PS II per mol photons (in the absence of repair) means that, for every 10^7 incident photons, one member among a collection of PS II complexes is inactivated. Note that this does not necessarily mean that *each* PS II complex is inactivated after it has processed 10^7 photons.

At a quantum yield of photoinactivation of 0.1 μmol PS II per mol photons (hv) 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 μmol photons m^{-2} s^{-1}) and assuming a typical initial content of 1 μmol functional PS II m^{-2}, the maximum gross rate of photoinactivation is 500 × 10^{-6} mol hv m^{-2} s^{-1} × 10^{-7} mol PS II (mol hv)$^{-1}$ × 3600 s h^{-1}/10^{-6} mol PS II m^{-2}=18 % h^{-1}

As the content of functional PS II decreases, there are few PS II complexes available for photoinactivation, and the rate of photoinactivation declines [60]. Nevertheless, on one or two sunny days, the entire population of PS II complexes may have to be repaired by *de novo* synthesis and incorporation of new D1 protein.

The probability of photoinactivation of PS II

To a certain extent, the photoinactivation of PS II 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 (1) PS II complexes in a homogeneous population are photoinactivated independently, at least in the early to mid-

dle stages of the population decline (see later), and (2) 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 the number of functional PS II complexes y should decrease exponentially with photon exposure from an initial value y_o [41]:

$$y = y_0 \exp(-kx)$$

The quantum yield of photoinactivation at a given value of y is the slope at that point, and should not be confused with p, 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 [42, 79].

Possible chemical candidates responsible for photoinactivation of PS II

Hydroxyl free radicals

In an atmosphere containing oxygen, electron transfers inevitably lead to the formation of some superoxide anion radicals:

$$O_2 + e \rightarrow R\ O_2^-$$

Superoxide anions are not very reactive radicals, no more reactive than oxygen itself, but superoxide dismutase (SOD) catalyses a reaction to form H_2O_2 which is much more reactive and dangerous:

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

H_2O_2 can undergo one further reaction to form the hydroxyl radical, either by reaction with a reduced iron centre or, more probably, with another superoxide anion:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\cdot} \quad \text{or}$$
$$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + HO^{\cdot}$$

$HO^{\cdot}$ is the most reactive of the three radicals, being able to modify proteins so as to make them susceptible to proteolytic attack [16]. Its formation must, therefore, be prevented. Leaves contain enzymes (ascorbate peroxidase, monodehydroascorbate peroxidase, dehydroascorbate peroxidase and glutathione reductase) which help to convert H_2O_2 to H_2O, while regenerating the electron donors [1, 9, 10]. Provided these enzyme systems function adequately, reactive oxygen species will not contribute to the photoinactivation of PS II. Tyystjarvi *et al.* [81] observed only a slight photoprotective effect of over-expressing glutathione reductase in tobacco cv. Samsun and none in the cv. Bel W3. Presumably, the wild-type already possesses adequate levels of anti-oxidative enzymes. Indeed, when the concentration of oxygen is increased to 60%, photoinactivation of PS II in pea leaves is *ameliorated* (due to utilization of excess light by the Mehler reaction, and the dissipation of excitation energy via a pH gradient promoted by the Mehler reaction) despite the increased production of O_2^- [63].

Under normal circumstances, it seems reasonable to conclude that reactive oxygen species are safely disposed of. In some conditions, such as in the case of chilling-sensitive plants exposed to low temperatures, reactive oxygen species may constitute a problem. When that happens, PS I, the predominant site of production of O_2^-, may be photoinactivated before PS II [34, 74, 75, 77].

Singlet oxygen, $^1O_2$

The reaction centre of PS II is a shallow trap; excitation energy trapped by the reaction centre and inducing an electron transfer from a special chlorophyll (P680) to pheophytin (Ph), may re-emerge when charge recombination occurs in the radical pair, $P680^+\ Ph^-$. This is the basis of the exciton/radical pair equilibrium model [69, 71, 78]. If the radical pair is in a singlet state, $^1[P680^+\ Ph^-]$, where the two unpaired electrons have anti-parallel spin, charge recombination will produce singlet excited $^1P680^*$:

$$^1[P680^+\ Ph^-] \rightarrow {}^1P680^* + Ph$$

On the other hand, a triplet state of the radical pair, in which the two unpaired electrons have parallel spin, will give triplet P680:

$$^3[P680^+\ Ph^-] \rightarrow {}^3P680^* + Ph$$

Triplet P680, having a relatively long lifetime, may react with ordinary triplet oxygen to give singlet excited oxygen:

$$^3P680^* + {}^3O_2 \rightarrow P680 + {}^1O_2$$

Singlet oxygen is highly reactive. Once formed, it should be removed quickly if it is not to damage the photosynthetic apparatus. Carotenoids play a role in de-activating singlet oxygen:

$$^1O_2 + {}^1Car \rightarrow {}^3Car + {}^3O_2$$
$$^3Car \rightarrow {}^1Car + heat$$

The alternative possibility that carotenoids could prevent the formation of singlet oxygen by dissipating $^3P680^*$ does not seem to hold in practice because β-carotene in the reaction centre must be at a safe distance from $P680^+$, the strongest oxidant in photosynthesis [11]. Nevertheless, under conditions where charge recombination is expected to be enhanced, namely, during continuous illumination when the primary quinone acceptor (Q_A) is chemically reduced and forward electron transfer from Ph^- to Q_A cannot occur, it has been suggested that the decay of $^3P680^*$ is hastened by a hundred-fold [82]. If so, singlet oxygen production is min-

imized under such conditions, and the ever-occurring photoinactivation of PS II is less likely to be caused by singlet oxygen *in vivo*. Further, it has been proposed that the site of location of P680 in a *functional* PS II complex is not freely accessible to oxygen molecules, and that there is a specific channel to direct O_2 liberated from water molecules out to the membrane surface, thereby restricting the formation of singlet oxygen [2]. A similar exclusion of oxygen from the site of production of triplet P700 has also been proposed for the PS I complex [72]. Thus, although it has been reported that singlet oxygen is produced in broad bean leaves [30], the rate of production is proportional to the non-functional PS II complexes; presumably, although structurally perturbed *non-functional* PS II complexes may allow oxygen access to P680, *functional* PS II complexes are not inactivated by singlet oxygen in the first place.

$P680^+$

$P680^+$, the most powerful oxidant in photosynthesis, is formed when an electron is transferred from P680 to pheophytin (Ph). $P680^+$ is ultimately required to extract electrons from otherwise stable water molecules. Anderson *et al* [4]. concluded that $P680^+$ is most probably the predominant chemical species that inadvertently inactivates PS II. Although direct evidence of photoinactivation by $P680^+$ is difficult to obtain, there are a number of indirect lines of evidence for the case [4], including:

(a) While Ph^- quickly transfers its electron to Q_A within about 10 ps after light is absorbed, $P680^+$ does not obtain its replacement electron for 20-200 ns. Therefore, $P680^+$ exists for a relatively long time during which it may react with its surroundings; and

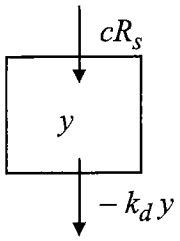
(b) Given that exciton-radical pair equilibrium holds, a photon visits a PS II reaction centre and causes primary charge separation (electron transfer from P680 to Ph); then charge recombination between $P680^+$ and Ph^- occurs, yielding an exciton in the antenna. In this way, an exciton may visit a reaction centre several times before being finally trapped [71]. 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 $P680^+$ and therefore to possible photoinactivation of PS II. This may be a basis for the dependence of photoinactivation on photon exposure, and the reciprocity law as mentioned above.

The variability of photoinactivation of PS II: two of the mitigating factors

Plants have evolved numerous strategies which minimize the photoinactivation of PS II [for reviews see 7, 17, 45, 49, 56, 67]. Briefly, these include adjustment of leaf orientation [35, 43, 52], chloroplast movement as a light-avoidance response [20, 62], scavenging of reactive oxygen species [9], photorespiration as a safety valve [36, 55, 57, 66], the Mehler reaction [62,68], transthylakoid (pH-dependent quenching of excitation energy [25, 38, 47, 58], dissipation via zeaxanthin in the xanthophyll cycle [14, 21, 26, 27], photosynthetic acclimation to high light [see 17 and references therein) and adjustment to a smaller antenna [64]. In the following sections, two photoprotective strategies are singled out for discussion, namely, *de novo* synthesis of D1 protein and a novel protection of functional PS II complexes by photoinactivated neighbours.

Recovery from photoinactivation via *de novo* synthesis of D1 protein

Recovery from light-induced damage requires the synthesis of the D1 protein, as shown in *Chlamydomonas* [40, 50], *Anacystis* [70], pea [51], and beans [28]. Protein synthesis can be monitored by the incorporation of radiolabelled aminoacids into proteins [5, 24, 44]. Using leaves of *Brassica napus*, Sundby *et al.* [76] conducted a detailed study of the parallel synthesis and degradation of the D1 protein in PS II by measuring both (1) the net incorporation of radiolabel as a function of irradiance at a fixed duration (t =1 h), and (2) D1 protein degradation as revealed by the exponential loss of radiolabel in a pulse-chase experiment. The data of Sundby et al. [76] can be used to derive the gross rate of D1 protein synthesis as a function of irradiance, as follows.



Let the gross rate of D1 protein synthesis be R_s, the fraction of radiolabelled aminoacid be c, the concentration of radiolabelled PS II complexes be y and the rate coefficient for D1 degradation be k_d. The net rate of increase of y is

$$dy/dt = cR_s - k_d y$$

Since at $t = 0$, $y = 0$, the solution of this equation gives

$$R_s = yk_d / [c\ (1 - \exp(-k_d t)] \quad (1)$$

The values of k_d at various irradiances are obtained from Fig. 6 of Sundby *et al.* [76], t is the duration of radiolabelling (1 h) and y is obtained in relative units from the lower curve in Fig. 5 of Sundby *et al.* [76] to give, in arbitrary units, the gross rate of D1 protein synthesis R_s [given by equation (1) above] as shown in Fig. 3 below. A notable feature of the graph is that the gross rate of D1 protein synthesis in *Bras-*

sica napus increases with irradiance up to about 350 μmol m^{-2} s^{-1}, the irradiance used to grow the plants. Above this irradiance, the gross rate of synthesis declines with irradiance. An independent method also confirmed that the rate coefficient for D1 protein synthesis in capsicum leaves declines with high irradiance (> growth irradiance, Lee *et al.* 2001). It seems surprising that D1 protein synthesis should decline at high irradiance just when the fast repair of the photodamage to PS II is most needed. One possible explanation [42] is that the stromal pH at high irradiance is beyond the narrow range (pH 7.6-7.9) [13] optimal for D1 protein synthesis.

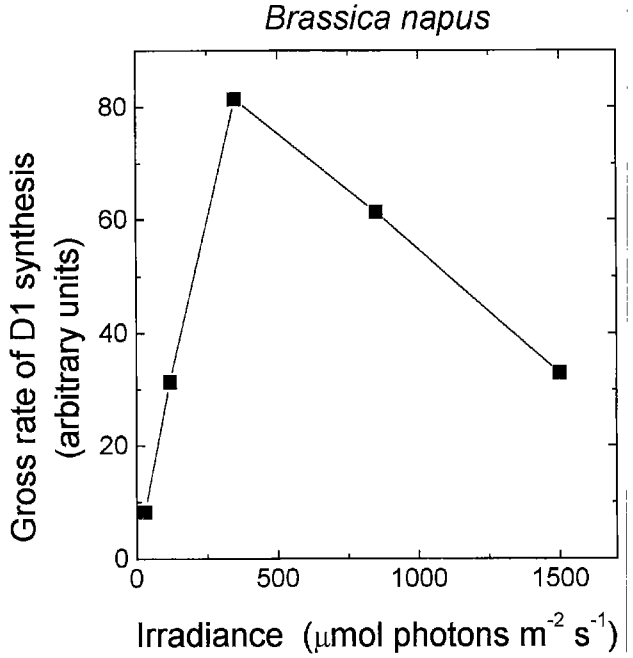
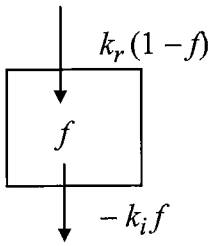


Fig. 3. The dependence of the gross rate of D1 protein synthesis on irradiance. Data are re-calculated (as explained in the text) from Figs. 5 and 6 of Sundby *et al.* (1993) for *Brassica napus* grown under moderate irradiance (350 μmol m^{-2} s^{-1}). Control leaf discs (without any pre-photoinhibitory treatment) were illuminated at varied irradiance.

Given that the gross rate of D1 protein synthesis first increases and then decreases as irradiance is increased, at least in some species, one can hardly expect reciprocity to hold under conditions where the rate of D1 protein synthesis changes with irradiance. Indeed, as shown in Fig. 2B, capsicum leaf discs in the absence of lincomycin and in normal air do not exhibit reciprocity of irradiance and duration of illumination. Only in the presence of lincomycin (Fig. 2A) or of high CO_2 (Fig. 2C, at 460 and 1800, but not 900, μmol photons m^{-2} s^{-1}) did we observe reciprocity.

Optimal recovery occurs at low irradiance

Given that the gross rate of D1 protein synthesis is maximal at about the growth irradiance, one expects the optimal irradiance for recovery to be at or below the growth irradiance. This is because the rate coefficient for photoinactivation increases linearly with irradiance [42, 79], and higher irradiances are unfavourable for recovery. To model the time-course of recovery at various irradiances, one needs to know k_r, the rate coefficient for repair (which, when multiplied by the concentration of inactive PS II complexes to be repaired, gives the gross rate of D1 protein synthesis). However, apart from a study by Lee *et al.* [42], such values are rarely reported. Therefore, we will attempt to determine what assumed values of k_r will give time courses of recovery that are consistent with observations. Let f be the fraction of functional PS II complexes, k_r be the rate coefficient for D1 protein synthesis and k_i be the rate coefficient for photoinactivation of PS II.



The net rate of change of f with t is

$$df/dt = k_r (1 - f) - k_i f$$

For a specific example of conditions in which at $t = 0$ (beginning of recovery), $f = 1/2$, the solution of this equation is

$$f = k_r/(k_i+k_r) + (k_i+k_r)\exp[-(k_i+k_r)t]/(2k_r) \quad (2)$$

Assume that k_i is directly proportional to irradiance [42, 79]; the constant of proportionality can then be estimated from Line 2 (growth irradiance 250 μmol m^{-2} s^{-1}) of Table II of Aro *et al.* [6] to be 5.4×10^{-4} h^{-1} m^2 s mol^{-1}, a value similar to that obtained for capsicum leaves [42]. The constant of proportionality in turn can be used to calculate k_i at any required irradiance. Next, we assume k_r to be 0.45, 0.5, 0.65, 0.8 and 0.7 h^{-1} at recovery irradiances 20, 50, 200, 400 and 1000 μmol m^{-2} s^{-1}, respectively. These assumed values of k_r are comparable to those found at 25°C for capsicum grown under 100 μmol m^{-2} s^{-1} [42]. From k_i and k_r one can calculate the time course of the recovery of f according to equation (2) (Fig. 4).

Aro *et al.* [8] reported the time-course of recovery from photoinactivation of PS II in pea leaves, as measured by the chlorophyll fluorescence ratio F_v/F_m. Qualitatively, the pattern of dependence of recovery on the irradiance reported by Aro *et al.* [8] is consistent with the predictions of Fig. 4 based on assumed values of. k_r. However, this consistency between the measurements of Aro et al. [8] and the predictions of Fig. 4 are conditional on (1) the ratio F_v/F_m being a linear measure of the functional PS II content, and (2) the

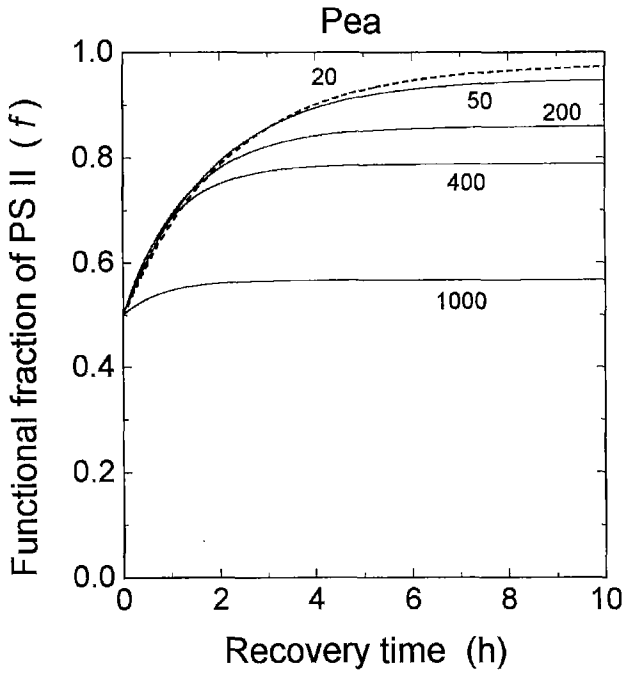


Fig. 4. Calculated time course of changes in the fraction of functional PS II during recovery of photoinactivated PS II in pea leaves at varied irradiances: 20, 50, 200, 400 or 1000 μmol m^{-2} s^{-1}. The curves are calculated according to equation (2) as described in Section 3.1.1, assuming values of k_r and k_i as explained in the text. It is further assumed that half of the PS II complexes are non-functional at the beginning of the recovery period.

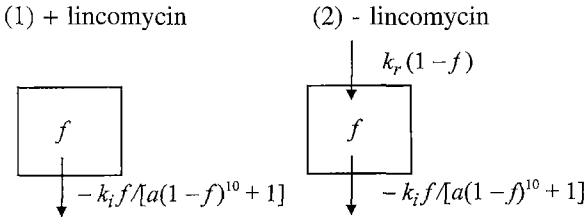
assumed values of k_r being realistic. In relation to condition (1), it is known that the ratio Fv/Fm is not a linear measure of functional PS II in pea leaves [61]. Further, the kinetics of recovery from photoinactivation of PS II needs to be better characterized, not just by measurement of chlorophyll fluorescence, but also by O_2 evolution. Chow *et al.* [19], for example, showed that these two measurements show rather different recovery kinetics after high-light treatment of spinach leaves. In relation to (2), there is an obvious need to devise a method for determining k_r experimentally. The method of Lee *et al.* [42] is not easily applicable to low irradiances. In general, the kinetics of recovery from high-light stress merit further detailed investigation.

Photoinactivated PS II complexes protect their functional neighbours

Krause [37] hypothesized that a photoinactivated PS II complex may dissipate excitation energy efficiently as heat, thereby preventing further damage to itself, e.g. destruction of antenna pigments. Öquist *et al.* [54] took this hypothesis further by proposing that a photoinactivated PS II complex, by acting as an energy sink, may protect neighbouring, connected functional PS II complexes from photoinactivation. Initial attempts to test this hypothesis [41, 80] did not uncover evidence consistent with predictions of the hypothesis. Later, Lee *et al.* [42] extended the light treatment of capsicum leaf discs to large photon exposures which decreased the functional fraction of PS II to 30% or less, and observed a resilient population which survived large photon exposures despite the inhibition of D1 protein synthesis by lincomycin. The residual sub-population of PS II is depicted in the semi-log plot in Fig. 2A. It is seen that the decline in functional PS II is mono-exponential until about 30% of functional PS II remains; then deviation from a single exponential decline occurs abruptly, leaving a relatively constant fraction of functional PS II. A similar observation was also made in low-light grown capsicum [42] and high-light grown pea [64]. I propose that this mode of photoprotection, which kicks in after the majority of PS II complexes have been photoinactivated, be named "last-ditch photoprotection".

A simple kinetic model incorporating last-ditch photoprotection

Lee *et al.* [42] proposed that a small residual sub-population of functional PS II is protected by photoinactivated neighbours. They considered two cases: (1) only photoinactivation occurs in the presence of lincomycin and (2) both photoinactivation and repair occur simultaneously in the absence of lincomycin:



As Fig. 2A indicates, the decline in functional PS II with time (when the irradiance is fixed) is initially mono-exponential, but abruptly deviates from an exponential decline after about 70 % of PS II complexes have been photoinactivated. In order to model the decline of functional PS II in lincomycin-treated capsicum leaf discs with illumination time, it was necessary to postulate that the "intrinsic" rate coefficient k_i is significantly modified by a factor $1/[a(1-f)^{10}+1]$ when more than about 70% of the PS II complexes have been photoinactivated; at a lesser extent of photoinactivation, the factor has a negligible effect on k_i. The factor $1/[a(1-f)^{10}+1]$ is a purely formal description of an effect that comes into operation only after a heavy loss of functional PS II complexes. Solving the above two cases simultaneously for k_i and k_r [42] requires an appropriate value of a, which was chosen by trial and error to give a good fit to the data in Case (1). At a given functional fraction of PS II, the effectiveness with which photoinactivated PS II complexes protect their functional neighbours is indicated by the value of a: the larger the value, the more effective is the mechanism. Studying how the value of

a varies with experimental conditions then gives an indication of what is required to operate this photoprotective mechanism.

Apparent requirement for a ΔpH to operate the last-ditch photoprotective mechanism

Table 2 shows values of *a* obtained for illumination of capsicum leaf discs in the presence or absence of nigericin. It is seen that *a* is much smaller when nigericin is present. As nigericin is an uncoupler that abolishes the transthylakoid Δ pH, it appears that a high value of *a* requires a large ΔpH. That is, the effectiveness with which photoinactivated PS II complexes protect their functional neighbours appears to depend on a ΔpH.

Table 2. Summary of values of *a* (a measure of the effectiveness with which photoinhibited PS II complexes protect their functional neighbours), estimated as described in the text. When used, [nigericin] = 5 μM. Values with an asterisk are from Lee *et al.* (2001), while values obtained in the presence of nigercin are from Lee, Hong and Chow (submitted).

Growth irradiance	Treatment irradiance (μmol photons m^{-2} s^{-1})	Chemical treatment	*a*
Low			
(a) 100	1800	+ H_2O	50*
(b) 100	1800	+nigericin	5
High			
(c) 500	1800	+ H_2O	50*
(d) 500	1800	+nigericin	0.5

Interestingly, when thylakoids are isolated from pea leaves and subjected to photoinhibitory light treatment in the presence of nigericin, the decline in functional PS II is strictly mono-exponential [83]. The mono-exponential decrease in functional PS II means that for every active PS II the probability of photoinactivation in unit time (at a given irradiance) is constant throughout the light treatment and independent of the concentration of photoinactivated PS II units [41, 83]. This is expected to be the case according to our present kinetic model if $a \approx 0$ due to nigericin lowering the trans-thylakoid (pH, i.e. photoprotection of functional PS II by photoinactivated neighbours requires a ΔpH to operate.

It is not clear how an acidic lumen or a trans-thylakoid (pH can regulate the last-ditch photoprotective mechanism. However, protonation of proteins can certainly have profound effects on, for example, energy-dependent quenching [26, 32] and calcium-binding to PS II [39]. Horton *et al.* [32] have described four targets of protonation, each capable of regulating energy flow in PS II. 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 [46]. In this context, it is interesting to note that grana formation allows a controlled dissipation of excitation energy [31]: 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 profound effects of protonation on the PS II core and its peripheral chlorophyll *a/b* complexes, one expects that an acidic lumenal pH may play an central role in the last-ditch photoprotective mechanism. For example, an acidic lumenal pH may render photoinactivated PS II complexes strongly quenching, while also promoting the connectivity among PS II neighbours. Then, excitation energy absorbed into functional PS II complexes will be readily shunted to connected, non-functional PS II neighbours, which serve as strong sinks.

Concluding remarks

The photoinactivation of PS II complexes is an inevitable event in oxygenic photosynthesis using water as a substrate. $P680^+$ is required to oxidize water, but may inadvertently photoinactivate PS II. The photoinactivation of PS II depends on the dosage of light, and therefore, occurs even in weak light given over a longer time. The exact dependence of PS II photoinactivation, 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. Continual repair is necessary if net loss of function is to be avoided. Such repair requires *de novo* D1 protein synthesis, which may have a complicated dependence on the prevailing irradiance. To minimize photoinactivation of PS II, plants have evolved numerous photoprotective strategies. An interesting, possibly "last-ditch" strategy is the photoprotection of functional PS II complexes by photoinactivated neighbours; this mechanism appears to operate only when the majority of PS II 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 role in the recovery of photoinactivated PS II units when favourable conditions return - a kind of mutual aid and cooperation.

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