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Photoinactivation of photosystem II complexes and photoprotection by non-functional neighbours in *Capsicum annuum* L. leaves

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Abstract. Leaf segments from *Capsicum annuum* plants grown at 100 μmol photons m^{-2} s^{-1} (low light) or 500 μmol photons m^{-2} s^{-1} (high light) were illuminated at three irradiances and three temperatures for several hours. At various times, the remaining fraction (*f*) of functional photosystem II (PS II) complexes was measured by a chlorophyll fluorescence parameter ($1/F_o - 1/F_m$, where F_o and F_m are the fluorescence yields corresponding to open and closed PS II traps, respectively), which was in turn calibrated by the oxygen yield per saturating single-turnover flash. During illumination of leaf segments in the presence of lincomycin, an inhibitor of chloroplast-encoded protein synthesis, the decline of *f* from 1.0 to about 0.3 was mono-exponential. Thereafter, *f* declined much more slowly, the remaining fraction (≈0.2) being able to survive prolonged illumination. The results can be interpreted as being in support of the hypothesis that photoinactivated PS II complexes photoprotect functional neighbours (G. Öquist et al. 1992, Planta 186: 450–460), provided it is assumed that a photoinactivated PS II is initially only a weak quencher of excitation energy, but becomes a much stronger quencher during prolonged illumination when a substantial fraction of PS II complexes has also been photoinactivated. In the absence of lincomycin, photoinactivation and repair of PS II occur in parallel, allowing *f* to reach a steady-state value that is determined by the treatment irradiance, temperature and growth irradiance. The results obtained in the presence and absence of lincomycin are analysed according to a simple kinetic model which formally incorporates a conversion from weak to strong quenchers, yielding the rate coefficients of photoinactivation and of repair for various conditions, as well as gaining an insight into the influence of *f* on the rate coefficient of photoinactivation. They demonstrate that the method is a convenient alternative to the use of radiolabelled amino acids for quantifying photoinactivation and repair of PS II in leaves.

Key words: *Capsicum* (photoprotection) – Chlorophyll fluorescence – Photoinactivation – Photoinhibition – Photoprotection – Photosystem II

Abbreviations: D1 protein = *psb*A gene product; *f* = functional fraction of PS II complexes; F_o, F_m = minimum and maximum chlorophyll fluorescence yield corresponding to open and closed PSII traps, respectively; F_v = variable chlorophyll fluorescence; k_i, k_r = rate coefficient of photoinactivation and repair, respectively; P680 = special chlorophyll pair in the PS II reaction centre; PS II = photosystem II; y, y_o = the concentration of functional PS II after and before a light treatment, respectively

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Introduction

During normal photosynthesis, even in low light, photoinactivation of photosystem II (PS II) and repair via de-novo protein synthesis (particularly that of D1 protein) occur in parallel (for reviews, see Prásil et al. 1992; Aro et al. 1993; Chow 1994; Osmond 1994; Melis 1999). If repair is able to completely keep pace with photoinactivation, no net loss of functionality of PS II is observed. When chloroplast-encoded protein synthesis is prevented, however, loss of PS II function is readily revealed: in bright room light in the presence of lincomycin, each square mm of leaf may lose 10^6 functional PS II complexes per second (Lee et al. 1999), the rate increasing with irradiance. Thus, excess light may still lead to a net loss of PS II activity even when repair can take place. Photoinactivation of PS II is thought to be due to the strongest oxidant known in photosynthesis, $P680^+$, which is needed to ultimately oxidise water, but which can also oxidise other neighbouring groups with a small but not insignificant probability (Thompson and Brudvig 1988; Barber 1995; Anderson et al. 1998).

It has been hypothesized that a photoinactivated PS II may dissipate excitation energy efficiently as heat,

preventing further damage to the complex itself, e.g. gross destruction of antenna pigments (Krause 1988). Öquist et al. (1992) further hypothesized that photoinactivated PS II complexes acting as sinks for excitation energy may help to prevent neighbouring, connected PS II complexes from photoinactivation. They based this conclusion on the observation that the sum of energy-dependent quenching (qN_e) and slowly reversible quenching (qN_i) of chlorophyll fluorescence in light-stressed leaves is approximately equal to qN_e of control leaves in which qN_i is absent. Apparently, light-stressed leaves, although possessing a diminished capacity for qN_e, were able to quench excitation energy partly via qN_i. However, attempts to find evidence for the hypothesis of Öquist et al. (1992) have not yet been successful. Tyystjärvi et al. (1994), for example, observed in spinach thylakoid preparations only a single exponential decline in PS II functionality with time; if non-functional PS II complexes were able to help connected, functional neighbours dissipate excitation energy, the time-course should have deviated from a mono-exponential decline. Our own study (Lee et al. 1999) also showed that the decline in functional PS II seemed to be describable by a single exponential function of the light dose, although the data were rather scattered, and the photon exposures used were not excessive.

The present study re-examines in detail the response of *Capsicum* leaf discs to light as a function of illumination time, at various irradiances and temperatures. The results, apart from providing an insight into the dependence of the rate coefficients of photoinactivation and repair on various conditions, support the idea that photoinactivated PS II complexes help to photoprotect neighbouring, connected PS II units, but only after prolonged illumination when a substantial fraction of the PS II complexes has also been photoinactivated.

Materials and methods

Plant material and growth conditions

Capsicum annuum L. (cv. New Town No. 3) plants were grown in a potting mixture and watered daily with dilute Aquasol solution supplemented by iron chelate. The growth chamber was maintained at a 24/21 °C (day/night) regime, the growth irradiance being 500 µmol photons m^{-2} s^{-1} (high) or 100 µmol photons m^{-2} s^{-1} (low), supplied by a combination of metal-halide (MBID250/T/H, Kolorare, Hungary) and incandescent lamps. Leaves that had recently expanded fully were taken from plants at 30–40 d after sowing.

Uptake of lincomycin

Detached leaves were allowed to take up 3 mM lincomycin through the cut petiole in the dark for about 3 h. If on occasions the transpiration rate was too high, the leaf was covered with moist tissue paper. The average concentration of lincomycin taken into the tissue (2–3 mM) was estimated from the volume of solution taken up and the fresh weight of the leaf.

Light treatments

Leaf pieces (ca. 7 × 7 mm) were floated, adaxial side up, on distilled water or a solution of 1 mM lincomycin (for leaves that had taken up lincomycin) in a container kept at a defined temperature in a water bath. Illumination in normal air was provided at 460, 900 or 1,800 µmol photons m^{-2} s^{-1} by an HMI Universal Spotlight (Model HMI 575 W/GS; Osram) behind a heat filter (Schott 115, Tempax) and a piece of glass. Typically, 5 leaf pieces were sampled at each time-point, and 3 experiments were done to obtain a mean value and the standard error of the mean for 15 leaf pieces for each time point.

Measurement of functional PS II content by oxygen yield

The number of functional PS II complexes was determined in leaf discs (3.4 cm^2) according to Chow et al. (1991). Following an initial dark equilibration of 10 min, repetitive single-turnover, saturating xenon flashes (10 Hz, 2.5 µs full width at half peak height) were applied for 4 min, followed by 4 min darkness. This was followed by a second cycle of flashes and darkness. The slight heating artefact due to the flashes was taken to account, and limitation of linear electron transport by PS I was minimized by the use of background far-red light Chow et al. (1991). The number of PS II complexes was expressed on a chlorophyll basis, and normalized according to the control samples. Chlorophyll was assayed in buffered 80% acetone according to Porra et al. (1989).

Measurement of chlorophyll fluorescence yields

Leaf pieces were sampled after an illumination treatment and dark-adapted for 30 min before measurement of F_o (corresponding to open PS II reaction centres) and F_m (closed reaction centres) using a Plant Efficiency Analyser (Hansatech, King's Lynn, Norfolk, UK). Chlorophyll fluorescence yields after a light treatment (F_o and F_m) were all normalized to the F_o (set as 1.00) of leaf samples before illumination. Because of this normalization, $1/F - 1/F_m = F_v/F_m$ for control samples, and represents 100% of the functional PS II complexes present before any photoinhibitory treatment. This parameter has been suggested to be an indicator of PS II reaction centre functionality (Havaux et al. 1991; Walters and Horton 1993), and empirically correlated linearly with the oxygen yield per single-turnover flash (Park et al. 1995; Lee et al. 1999).

Imaging by chlorophyll fluorescence quenching

Interpretation of data from the above experiments presupposes that all cells and chloroplasts in the leaf tissue are exposed to similar levels of lincomycin (an inhibitor of chloroplast-encoded protein synthesis) and behave uniformly, at least with respect to chlorophyll fluorescence parameters. A portable chlorophyll fluorescence imaging system, described by Osmond et al. (1998), was therefore used to check the uniformity of fluorescence intensity and quenching in images captured at F_p (peak fluorescence yield at 1 s) and after 30, 90 and 300 s excitation (from red-light-emitting diodes, 1,300 µmol photons m^{-2} s^{-1}) of dark-adapted leaves. Controls were compared with leaves that had been pre-illuminated at 900 µmol photons m^{-2} s^{-1} and 15 °C in the presence of lincomycin to effect photoinactivation of PS II.

Results

Correlation of $1/F_o - 1/F_m$ with the oxygen yield per single-turnover flash

While the O_2 yield per single-turnover flash is a direct measure of the number of functional PS II complexes

capable of oxygen evolution in leaf discs (Chow et al. 1991), it is not a rapid method for assaying a large number of leaf discs. Therefore, we used the chlorophyll fluorescence parameter, $1/F_o - 1/F_m$, previously shown to be linearly correlated with the O_2 yield per single-turnover flash in pea (Park et al. 1995) and *Capsicum* (Lee et al. 1999) leaf discs that had been individually illuminated in moist air containing 1% CO_2. In the present study, leaf discs were floated on distilled water in normal air during illumination, with the adaxial side up; in this way, a large number of leaf pieces could be illuminated while being maintained at one of three temperatures. Figure 1 shows that the correlation between $1/F_o - 1/F_m$ and functional PS II still holds (closed symbols), and could be superimposed on the linear correlation between the two parameters (open symbols) obtained by Lee et al. (1999). Thus, the fractional decline in $1/F_o - 1/F_m$ is a measure of the fractional decline in functional PS II, and is used in this study in place of oxygen-yield measurements.

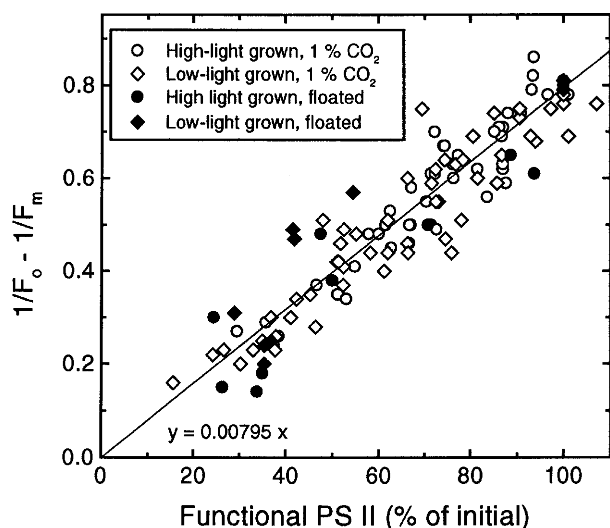

Fig. 1. Linear correlation of $1/F_o - 1/F_m$ with functional PS II content (measured by repetitive, saturating, single-turnover flashes) in leaf discs of high- or low-light-grown *Capsicum*. *Closed symbols* refer to leaf segments floated on distilled water (±pre-infiltration with lincomycin) in normal air during light treatment at 25 °C to bring about photoinactivation of PS II. *Open symbols* are data for leaf discs illuminated in 1% CO_2, re-plotted from Lee et al. (1999). The straight line is obtained by linear regression, constrained to pass through the origin

Decline in functional PS II in the presence of lincomycin

Lincomycin is often used to inhibit chloroplast-encoded protein synthesis (e.g. Aro et al. 1994). In the presence of lincomycin, therefore, photoinactivation of PS II takes place without repair. Figure 2 depicts the time course of the decline in functional PS II in leaf segments of low-light-grown (A) and high-light-grown (B) *Capsicum*. Leaves were previously allowed to take up lincomycin and the segments were also floated on a solution of lincomycin during illumination at one of three irradiances. Functional PS II as a fraction (*f*) of the initial amount declined faster as the treatment irradiance increased. In this semi-log plot, the straight-line decline in functional PS II complexes (down to $f \approx 0.3$) implies a random photoinactivation of seemingly independent PS II units. Thereafter, the decline was slower as the content of non-functional PS II units further increased. Finally, there remained a residual amount of functional PS II (about 20%) that seemed to survive illumination at 1,800 μmol photons m^{-2} s^{-1} for 5 h or more, even though no repair was taking place.

That this residual population did not seem to consist of chloroplasts that were inaccessible to lincomycin is supported by the following three experiments. Firstly, the inhibitor appeared to affect fluorescence quenching uniformly across a leaf disc, as shown in Fig. 3. In this figure, a control *Capsicum* leaf disc (dark-treated in the absence of lincomycin) gave the top row of colour maps, each of which shows the difference in fluorescence intensity between the images captured at F_p (at 1 s) and after 30, 90 or 300 s excitation. It is clear that a control leaf disc exposed to 1,300 μmol photons m^{-2} s^{-1} showed progressive quenching of chlorophyll fluorescence during illumination for up to 300 s. In a leaf disc that had been pre-illuminated in the presence of lincomycin (initially introduced through the petiole, followed by further uptake into leaf discs floating on a lincomycin solution) for 1 h at 900 μmol photons m^{-2} s^{-1} and 15 °C (middle row of images), the quenching (relative

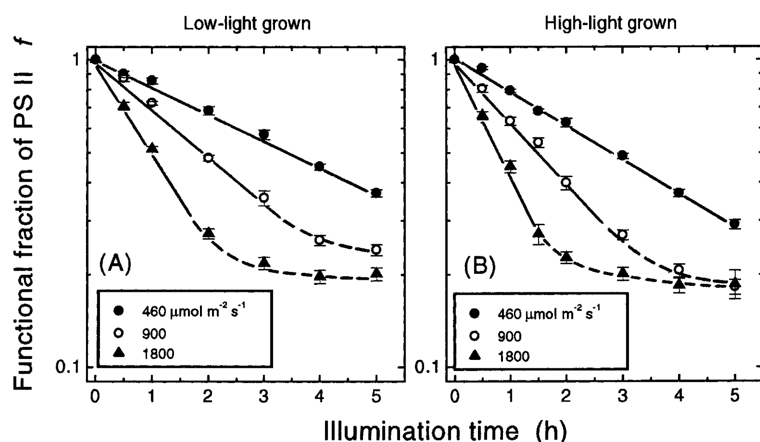

Fig. 2. Semilog plots of the decline in the fraction of functional PS II complexes in lincomycin-treated leaf segments of low-light-grown (**A**) or high-light-grown (**B**) *Capsicum*, as monitored by the normalized parameter $1/F_o - 1/F_m$ (see *Materials and methods*). Treatment irradiances were 460, 900 or 1,800 μmol photons m^{-2} s^{-1}. Each point is the mean ± SE of about 15 samples measured in three experiments (25 °C)

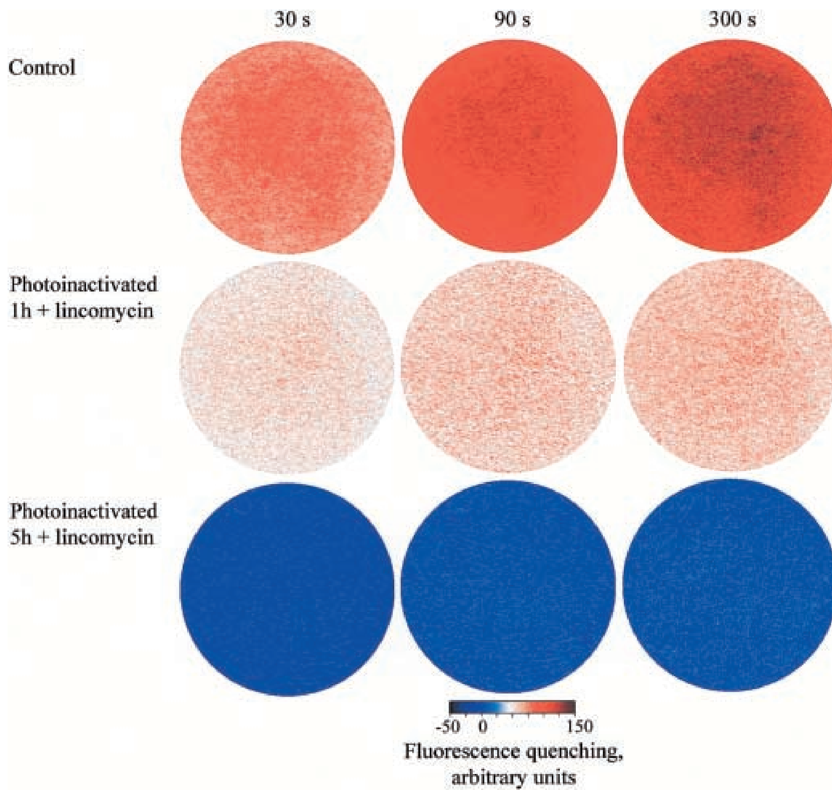

Fig. 3. Quenching of chlorophyll fluorescence in low-light-grown *Capsicum* control leaves (with neither light treatment nor lincomycin uptake; *top row*) or those pre-treated at 900 μmol photons m^{-2} s^{-1} and 15 °C for 1 (*middle row*) or 5 (*bottom row*) h in the presence of lincomycin. Colour maps show the difference in room-temperature fluorescence intensity between images captured at F_p (peak fluorescence at 1 s) and after 30, 90 and 300 s excitation of leaves (dark-adapted for 30 min prior to imaging) with red light (663 nm) from light-emitting diodes (1,300 μmol photons m^{-2} s^{-1}) using the imaging system of Osmond et al. (1998)

to F_p at 1 s) was much less but still uniform across the leaf surface. Pre-illumination for 5 h in the presence of lincomycin (bottom row) gave even smaller quenching relative to F_p at 1 s, but the quenching was still spatially uniform. The results suggest that the residual population of functional PS II was unlikely to be a proportion of centres that escaped exposure to lincomycin.

Secondly, vacuum infiltration of lincomycin (1 mM) into leaf segments was also used as a way of introducing lincomycin into leaf discs, perhaps more uniformly. After vacuum infiltration at 0.5 atmospheres of negative pressure for about 30 s, leaf discs were allowed to lose excess intercellular water by evaporation from the tissue in the dark for about 20 min. When such leaf segments were floated on a lincomycin solution (1 mM) and illuminated, a residual fraction of functional PS II again persisted even after prolonged illumination (data not shown). As vacuum infiltration was likely to give a uniform distribution of the inhibitor in all cells, the results suggest that the residual population persisted despite the presence of lincomycin.

Thirdly, to show that lincomycin was able to gain access to PS II complexes in general, we first illuminated leaf discs at 1,570 μmol photons m^{-2} s^{-1}, in the *absence* of lincomycin but at a lower temperature of 15 °C to hasten the photoinactivation of PS II. After 3.5 h illumination when about 15% of functional PS II units remained, leaf discs were vacuum-infiltrated with 1 mM lincomycin, allowed to lose excess intercellular water by evaporation, and then floated on 1 mM lincomycin at 25 °C and an irradiance of 15 μmol photons m^{-2} s^{-1}, in order to test for any recovery of PS II. As seen in Fig. 4, there was absolutely no recovery despite the favourable

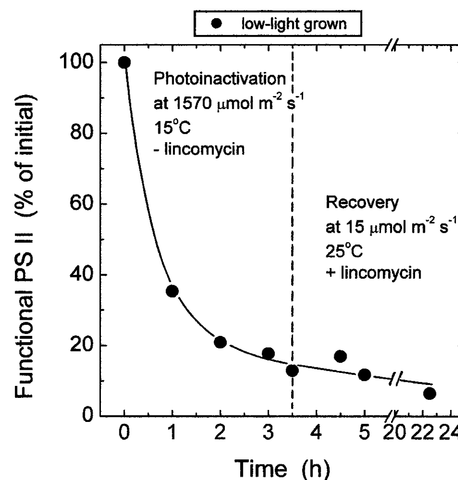

Fig. 4. Photoinactivation of PS II (quantified by the oxygen yield per repetitive single-turnover flash) during illumination at 1,570 μmol - photons m^{-2} s^{-1} and 15 °C in the absence of lincomycin for up to 3.5 h, and the subsequent lack of recovery in the presence of lincomycin, despite favourable temperature (25 °C) and irradiance (15 μmol photons m^{-2} s^{-1}) conditions

temperature and irradiance (Aro et al. 1994), showing that lincomycin was able to access all PS II complexes, thereby preventing any recovery.

Thus, it is likely that the residual population of functional PS II after prolonged illumination is photoprotected in some way, perhaps by an abundance of photoinactivated neighbours. To analyse the data obtained with lincomycin-treated leaf segments in terms of the hypothesis that photoinactivated PS II complexes photoprotect functional neighbours (Öquist et al. 1992),

we used a simple kinetic model [*Appendix* (i)]. The hypothesis predicts that as the population of photoinactivated PS II complexes builds up, its role as a sink for excitation energy becomes more important. Consequently, the rate coefficient for photoinactivation (equal to k_i when all PS II complexes are fully functional) is decreased by a certain factor, which is formally assumed in this model to be given by $1/[a(1-f)^{10} + 1]$, where a is a constant. This factor reduces to 1 when all PS II units are functional, but takes on a low value, $1/(a+1)$, when all PS II units are photoinactivated. In the absence of a mechanistic model, the choice of this factor to describe changes in the rate coefficient of photoinactivation is purely formal and meant to reflect the expectation that the rate coefficient of photoinactivation is modified significantly only when a photoinactivated PS II is converted from a weak quencher to a strong quencher of excitation energy, i.e. after a substantial fraction of PS II units have also been photoinactivated. The variation of this factor with f is shown in Fig. 8 [*Appendix* (i)] for two values of a.

The simple kinetic model predicts that illumination time t is a function of f given by Eq. 1 in the *Appendix* (i), so in Fig. 5 the data were fitted by an equation of this form to yield the parameter k_i for a suitable value of a (for a discussion, see later).

Decline in functional PS II content in the absence of lincomycin

When both photoinactivation and repair of PS II occur, a steady state is reached where the two rates are equal. The fraction of functional PS II complexes did indeed decline to a steady value in each case in Fig. 6, where the treatment temperature was 25 °C. It is seen that the steady-state fraction of functional PS II (f_s) was lower at a higher treatment irradiance, and lower in low-light- than high-light-grown *Capsicum*, as expected. Provided the only effect of lincomycin was to inhibit repair, without any impact on the forward photoinactivation reaction, the value of f_s, together with k_i and a (obtained in the

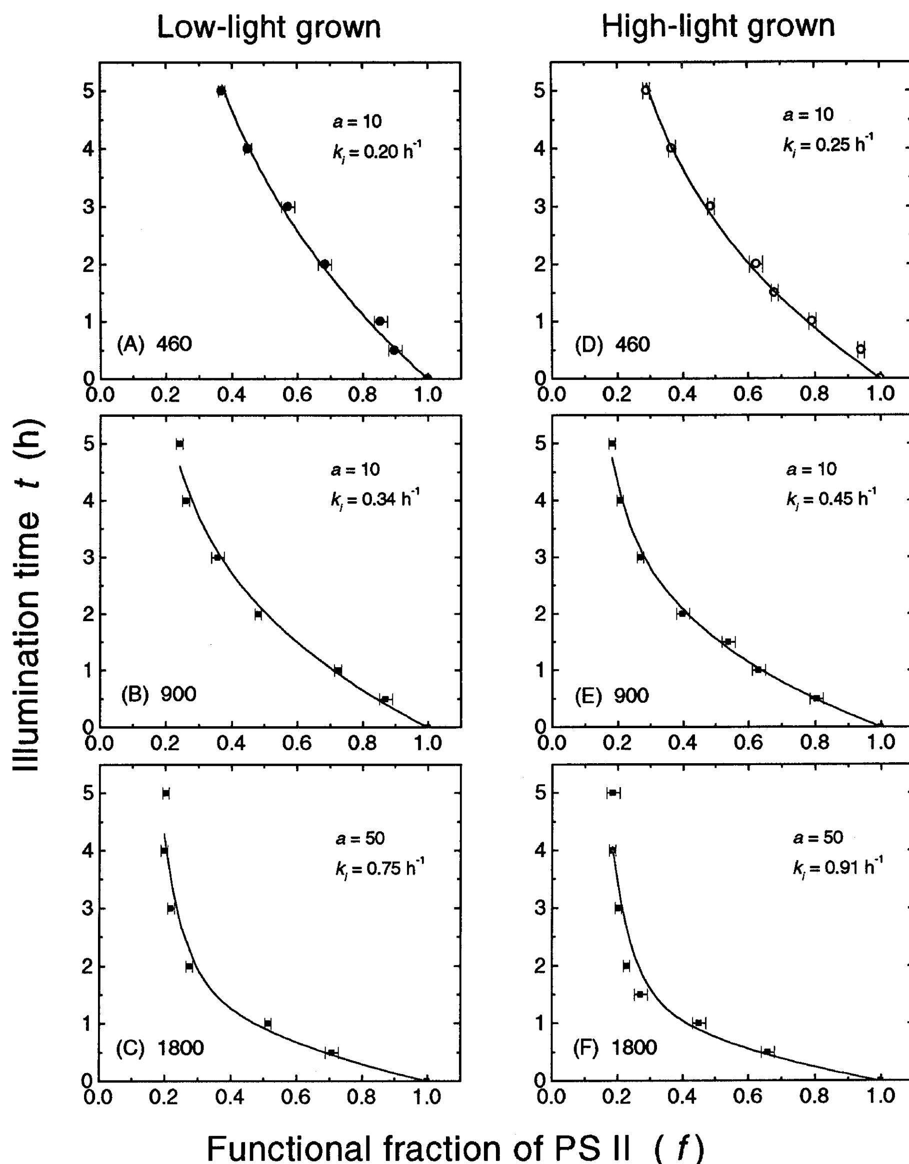

Fig. 5A–F. Relationship between illumination time (t) and the functional fraction of PS II (f) in *Capsicum* leaf segments during illumination at 460, 900 or 1,800 µmol photons m^{-2} s^{-1} at 25 °C in the presence of lincomycin. The data are fitted with Eq. 1 in the *Appendix* (i), using the values of k_i and a given in the panels (**A–C**, low-light-grown plants; **D–F**, high-light-grown plants)

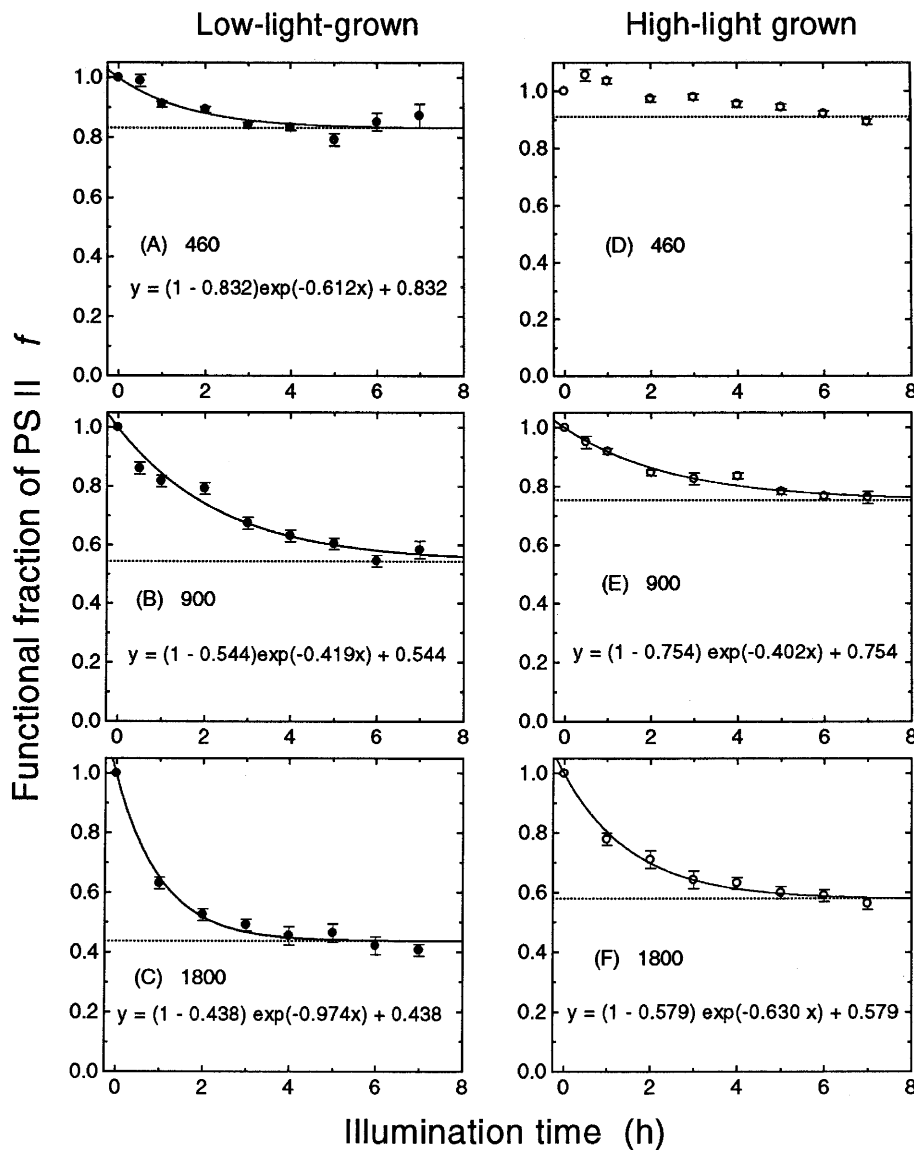

Fig. 6A–F. Time course of changes in the functional fraction of PS II (*f*) in *Capsicum* leaf segments during illumination at 460, 900 or 1,800 μmol photons m^{-2} s^{-1} at 25 °C in the absence of lincomycin. To estimate the steady-state values (f_s), the data are fitted with the curves for which the equations are given in the panels (**A–C**, low-light-grown plants; **D–F**, high-light-grown plants). *Horizontal dotted lines* indicate values of f_s

presence of lincomycin), determines k_r, the rate coefficient of repair, according to Eq. 2 in the *Appendix* (ii).

Variations in the rate coefficients of photoinactivation (k_i) and repair (k_r)

Analysis of data in Figs. 5 and 6 (25 °C) and at two other temperatures, using the kinetic model in the *Appendix* yields values of k_i and k_r. Figure 7 summarizes such values of k_i and k_r obtained for three temperatures, three treatment irradiances and two irradiances under which *Capsicum* plants were grown. At a low growth irradiance of 100 μmol photons m^{-2} s^{-1}, the rate coefficient of photoinactivation of PS II (k_i) increases with treatment irradiance (Fig. 7A). Similarly, k_i also increased with treatment irradiance for leaves from plants grown at the higher irradiance of 500 μmol photons m^{-2} s^{-1}, but with a greater slope. At each growth irradiance, k_i increased with temperature when leaves were exposed to 900 μmol photons m^{-2} s^{-1} (Fig. 7B). Interestingly, for a given treatment irradiance and temperature, k_i was marginally but consistently lower for plants grown at the lower irradiance (Fig. 7B).

At each growth irradiance, the rate coefficient for repair k_r decreased with treatment irradiance (Fig. 7C). Further, at a given treatment irradiance and 25 °C, k_r was considerably lower at the lower growth irradiance (Fig. 7C). The rate coefficient of repair (k_r) increased markedly with temperature, indeed by an order of magnitude when the temperature was increased from 15 to 35 °C.

Discussion

Photoinactivated PS II complexes help to photoprotect connected functional neighbours

The data can be interpreted according to the hypothesis that photoinactivated PS II complexes help to photoprotect connected functional neighbours (Öquist et al.

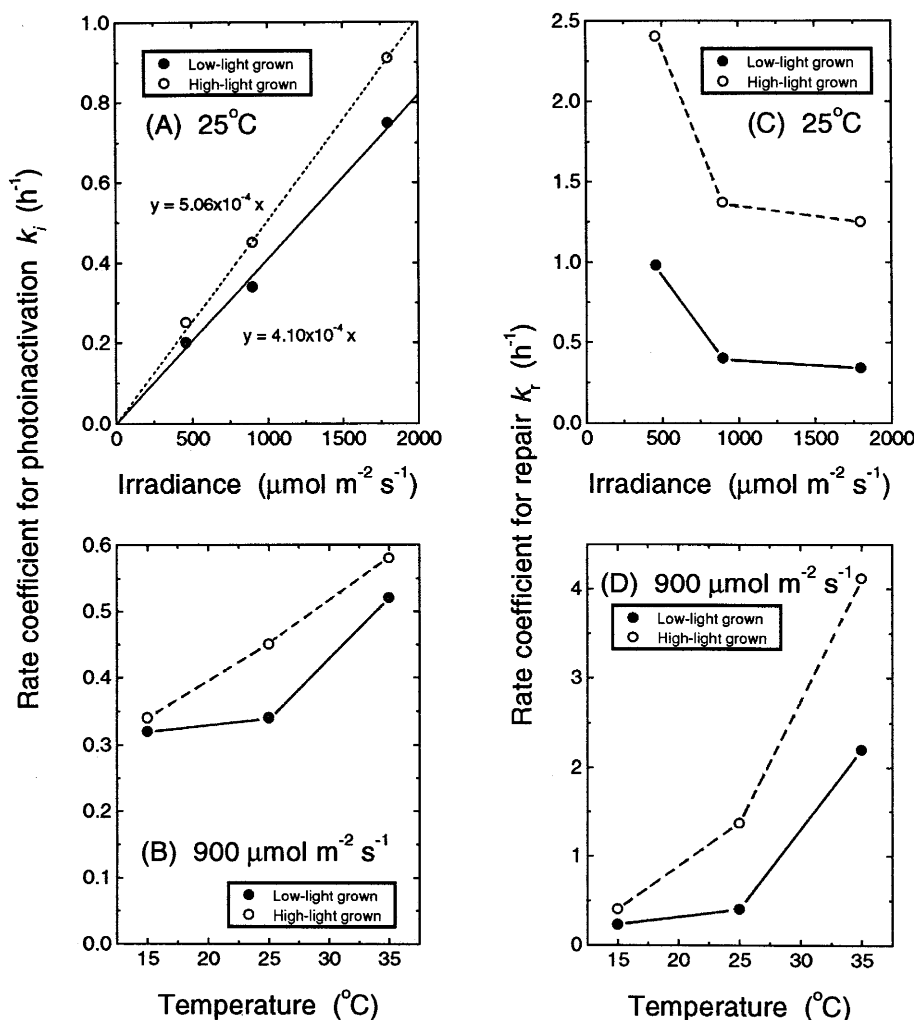

Fig. 7. Rate coefficients of photoinactivation (k_i) and of repair (k_r) as a function of treatment irradiance (**A, C**) or temperature (**B, D**), for low-light-grown (●) or high-light-grown (○) plants. The coefficients were estimated by curve fitting according to the *Appendix*, with $a = 10$ for 460 and 900 μmol photons m^{-2} s^{-1}, but $a = 50$ for 1,800 μmol photons m^{-2} s^{-1}

1992). Firstly, given that lincomycin seemed to access PS II complexes uniformly in leaf tissue (Figs. 3, 4), the retention of functional PS II complexes during prolonged illumination even in the presence of lincomycin (Fig. 2) implies that the remaining functional PS II units derive photoprotection from mechanisms other than repair. Secondly, the time course of decline in the fraction of functional PS II during illumination in the presence of lincomycin can be described by a simple kinetic model [*Appendix* (i)] in which the rate coefficient of photoinactivation k_i is modified according to the decreasing fraction of functional PS II (i.e. increasing fraction of non-functional PS II). Thus, as predicted by the hypothesis, the more abundant the non-functional PS II complexes, the greater is the degree of photoprotection. However, in order for the kinetic model to fit the data and yield values of k_i, it is assumed that any modification of the rate coefficient of photoinactivation is minimal until a substantial fraction of PS II complexes has been photoinactivated.

Rate coefficient of photoinactivation (k_i)

Tyystjärvi and Aro (1996) showed that the rate coefficient of photoinactivation of PS II in lincomycin-treated leaves is directly proportional to the treatment irradiance. Baroli and Melis (1996) also showed that the rate coefficient for the loss of D1 protein increased linearly with the irradiance under which *Dunaliella salina* cells were grown and measured. The linear increase in k_i with treatment irradiance (Fig. 7A), determined for both low-light- and high-light-grown *Capsicum*, is in agreement with these earlier findings. Thus, the range of values for k_i was similar to that observed for *Dunaliella* (Baroli and Melis 1996), and understandably somewhat greater than that observed for pumpkin (Tyystjärvi and Aro 1996) which was acclimated to a very high growth irradiance (1,000 μmol - photons m^{-2} s^{-1}). Interestingly, for a given increase in treatment irradiance, the increase in k_i was about 20% less in low-light-grown *Capsicum* than in high-light-grown plants. It is well known that low-light-grown plants have larger grana, and better connectivity between PS II units. Possibly, the better connectivity between functional PS II complexes and the dissipating, photoinactivated complexes plays a part in ameliorating photoinactivation, as has been hypothesized by Anderson and Aro (1994).

Interestingly, k_i was highest at 35 °C (Fig. 7B), i.e. higher temperatures promoted photoinactivation of PS II, in agreement with the earlier finding of Tyystjärvi et al. (1994) using isolated thylakoids. The higher temperature might have caused instability in the water-

oxidation complex, such that electron donation to $P680^+$ was slow. Under such conditions, the longer-lived $P680^+$, being predominantly responsible for photoinactivation (Anderson et al. 1998), could then damage other components of PS II with an increased probability.

Rate coefficient of repair (k_r)

The range of rate coefficients of repair in *Capsicum* (Fig. 7C,D), calculated by the present method, was comparable to values observed for D1 protein synthesis using [^{35}S]methionine labelling in *Dunaliella* (Vasilikiotis and Melis 1994), peas (Aro et al. 1994) and *Brassica* (Sundby et al. 1993).

As expected, k_r was highly dependent on temperature: it was very low at 15 °C but increased by an order of magnitude at 35 °C, both in low-light- and high-light-grown plants (Fig. 7D). The acceleration of repair at a higher temperature could be attributed to increased enzymic activity and faster temperature-dependent lateral diffusion of photoinactivated PS II, or parts of the entity, from granal stacks to stroma-exposed membrane regions where chloroplast ribosomes are located. There, de-novo synthesis of D1 protein occurs to replace the damaged protein (Hurewitz and Jagendorf 1987; Prásil et al. 1992; Aro et al. 1993).

Another general trend is a decrease in k_r with increase in treatment irradiance from 460 μmol photons m^{-2} s^{-1} upwards (Fig. 7C), implying slower rates of repair at higher irradiances. This is consistent with the results of Sundby et al. (1993) who reported that incorporation of radiolabelled methionine into D1 protein was already maximal at approximately the same irradiance as during growth, but that higher irradiances inhibited incorporation of radiolabel. There is not yet any clear explanation of why the rate coefficient for repair is lower at higher irradiances that are well above growth irradiance. One possibility is that at such high treatment irradiances, the stromal pH could be outside the narrow range of values (7.6–7.9) optimal for protein synthesis (Bhaya and Jagendorf 1984). In any case, the agreement between the two methods (the present one involving kinetic analysis and the other dealing with incorporation of radiolabelled amino acids into D1 protein) provides support of the present analysis as a valid alternative to radiolabelling experiments which may occasionally suffer from a non-uniform distribution of radiolabel in the leaf tissue (Russell et al. 1995).

Rate of turnover

Using the rate coefficients obtained from our analysis, we can estimate the absolute rate of turnover (photoinactivation and repair) of PS II complexes in the steady state. Consider, for example, high-light-grown *Capsicum*, exposed to a test irradiance of 900 μmol photons m^{-2} s^{-1} at 25 °C. Typically, the concentration of functional PS II in a control leaf is about 1 μmol PS II m^{-2} (see legend to Fig. 2 in Lee et al. 1999). Since $k_i = 0.45$ h^{-1} (Fig. 7A) and $f_s = 0.754$ (Fig. 6E), the rate of gross photoinactivation is

$$\begin{aligned} dy/dt &= -k_i y_s = -0.45\,h^{-1} \times 0.754 \times 1\,\mu mol\ PS\ II m^{-2} \\ &= -0.34\,\mu mol\ PS\,II\ m^{-2}\,h^{-1} \end{aligned}$$

This is equivalent to the inactivation of 10^7 PS II complexes per $(mm)^2$ per second. Since $k_r = 1.37$ h^{-1} (Fig. 7D), the rate of repair, being directly proportional to the concentration of photoinactivated PS II complexes, is

$$\begin{aligned} dy/dt &= k_r(y_o - y_s) \\ &= 1.37\,h^{-1} \times 0.246 \times 1\,\mu mol\ PS\ II\,m^{-2} \\ &= 0.34\,\mu mol\ PS\,II\ m^{-2}\,h^{-1} \end{aligned}$$

That is, the rate of turnover of PS II complexes in high-light-grown *Capsicum* under this set of conditions is 0.34 μmol PS II m^{-2} h^{-1} or about one-third of all PS II complexes every hour. For a comparison, using $k_i = 0.34$ h^{-1} (Fig. 7A), $k_r = 0.40$ h^{-1} (Fig. 7D) and $f_s = 0.554$ (Fig. 6B), the rate of turnover of PS II complexes in low-light-grown *Capsicum* under the same conditions is estimated to be 0.18 μmol PS II m^{-2} h^{-1}, about half the rate of high-light-grown plants.

Reciprocity of irradiance and duration of illumination

A number of studies have reported that during photoinactivation the reciprocity law is obeyed: an equal extent of PS II photoinactivation is effected by an equal dose of light (photon exposure; Bell and Rose 1981) when various combinations of the irradiance and duration of illumination are used (Jones and Kok 1966, Nagy et al. 1995; Park et al. 1995). Reciprocity is expected when PS II units are photoinactivated as independent units, with a *constant* probability of photoinactivation per unit photon exposure (Lee et al. 1999). Since the decline in functional PS II in the presence of lincomycin was mono-exponential until about 30% of functional PS II units are left (Fig. 2), reciprocity is expected to hold up to this extent of photoinactivation in the presence of lincomycin. However, reciprocity will not hold when more than 70% of the PS II units have been photoinactivated: the probability of photoinactivation per unit photon exposure is no longer constant owing to the possible photoprotective effect of a large, varying fraction of non-functional PS II units. In the absence of lincomycin, the decline in functional PS II levelled off much sooner, so reciprocity does not hold when there is no further net loss of functional PS II with time (and therefore, photon exposure).

Photoprotection by inactive PS II requires a large non-functional fraction of PS II complexes

Our previous study (Lee et al. 1999) involved a limited maximum extent of photoinactivation of PS II, so any residual population of PS II able to withstand continual

illumination was not apparent. Based on that study (Lee et al. 1999), it was postulated that a photoinactivated PS II is a relatively weak quencher compared with, for example, Chl_z^+ (Schweitzer and Brudvig 1997), able to dissipate only some excitation energy, notably that which it can no longer utilize by photochemical conversion. Why, then, is there much greater photoprotection when more than 70% of PS II units in both low-light- and high-light-grown *Capsicum* have been photoinactivated?

Possibly, in bringing about photoinactivation of a large fraction of PS II complexes, the large photon exposures required also convert the initially weakly quenching, inactive PS II complexes into strongly quenching centres. Presumably, photoinactived PS II units are continually being modified by oxidative reactions during sustained illumination. For example, since it is the *photoinactivated* PS II complexes that form singlet oxygen (Hideg et al. 1998), this highly reactive oxygen species may react locally to further modify the PS II complex in which it is generated. In addition, it has been shown that light-induced modifications of isolated PS II reaction centres include oxidative damage to D1 and other proteins in the reaction centre (Miyao 1994; Barber 1998). Further, reactive oxygen species may not be the only agent of chemical modification. Park et al. (1997) demonstrated a resistant fraction of PS II units (in pea leaf discs) that remained functional after prolonged illumination in anaerobic conditions and in the presence of lincomycin. Presumably, the modifying agent in anaerobic conditions is $P680^+$, the strongest oxidant in photosynthesis. Given these modification reactions, it is conceivable that rapid-quenching PS II centres may be produced during prolonged illumination. The great abundance of photoinactivated PS II centres after prolonged illumination also aided in photoprotection of residual functional centres. That such centres confer photoprotection of remaining functional PS II units may be extremely important for the survival of the leaf and, indeed, the plant.

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Appendix

A simple kinetic model of photoinactivation and repair of PS II

(i) Consider the case where repair of PS II is inhibited by lincomycin, and only photoinactivation occurs, converting functional PS II complexes (PS II_F, initial concentration y_o) into non-functional complexes (PS II_{NF}, concentration $y_o - y$), the remaining concentration of functional PS II at any time being y. The rate coefficient for photoinactivation is assumed to be non-constant, but consists instead of k_i multiplied by a factor that varies with the remaining functional fraction ($f = y/y_o$) of PS II

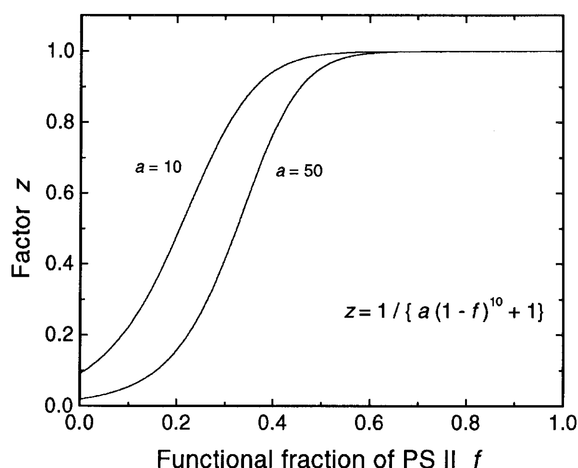
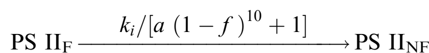


Fig. 8. Postulated factor $1/[a(1-f)^{10}+1]$ by which the rate coefficient of photoinactivation k_i is modified according to the functional fraction of PS II complexes f. The parameter a determines the lowest value of the factor at $f = 0$

complexes. Since the decline in f is mono-exponential until about 30% of functional PS II units remain (Fig. 2), we infer that any photoinactivated PS II complexes are only weak quenchers until that point. Thereafter, we assume that k_i is modified significantly by a factor determined by the non-functional fraction of PS II complexes, i.e. formally by a factor $1/[a(1-f)^{10}+1]$, where a is a constant:



$$\mathrm{PS\ II_F} \xrightarrow{k_i/[a\,(1-f)^{10}+1]} \mathrm{PS\ II_{NF}}$$

When all PS II complexes are functional ($f = 1$), the modifying factor reduces to 1. When all PS II complexes are non-functional, the factor assumes a low value $1/(a+1)$. The factor $1/[a(1-f)^{10}+1]$ varies with f as shown in Fig. 8 for two values of a.

During illumination in the presence of lincomycin when no PS II repair occurs, the rate of change of y with time is

$$dy/dt = -\{k_i/[a(1-f)^{10}+1]\}y \ .$$

Dividing by y_o and noting that $f = y/y_o$ the above equation can be rearranged to the form

$$df/dt = -k_i f/[a(1-f)^{10}+1]$$

or

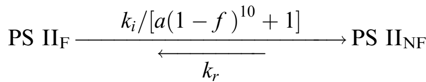
$$\int dt = -(a/k_i)\int\{[(1-f)^{10}+1]/f\}df \ .$$

Integration with the condition that at $t = 0, f = 1$ gives

$$\begin{aligned} t = &-(a/k_i)[\ln f - 10f + (45/2)f^2 - (120/3)f^3 \\ &+ (210/4)f^4 - (252/5)f^5 + (210/6)f^6 - (120/7)f^7 \\ &+ (45/8)f^8 - (10/9)f^9 + (1/10)f^{10} + 2.929] \\ &- (1/k_i)\ln f \end{aligned} \quad (1)$$

which can be fitted to a plot of illumination time (t) against the functional fraction of PS II (f) using a nonlinear curve fit program (Microcal Origin version 4.1), yielding the parameter k_i for a suitable value of a. Generally, a value of $a = 10$ gave a reasonable fit for data obtained at irradiances of 460 and 900 µmol photons $m^{-2}\ s^{-1}$, but a higher value of $a = 50$ was required to fit the data at 1,800 µmol photons $m^{-2}\ s^{-1}$.

(ii) Now consider the case in the absence of lincomycin where photoinactivation and repair (rate coefficient k_r) occur simultaneously. Assuming that the rate coefficient of photoinactivation is the same in the presence or absence of lincomycin,



As in (i), the rate of change in functional PS II with time of illumination is

$$dy/dt = -\{k_i/[a(1-f)^{10}+1]\}y$$

while the rate of repair is proportional to the concentration of photoinactivated PS II centres:

$$dy/dt = k_r(y_o - y)$$

At steady state, y is y_s, and the algebraic sum of these two rates is zero:

$$-k_i/[a(1-f_s)^{10}+1]y_s + k_r(y_o - y_s) = 0$$

Rearranging, and using $f_s = y_s/y_o$:

$$k_i f_s/[a(1-f_s)^{10}+1] = k_r(1-f_s) \qquad (2)$$

The ratio $f_s = y_s/y_o$ is determined experimentally under each set of conditions (e.g. Fig. 6). From the values of a, k_i and f_s, we obtain k_r using Eq. 2. The above analysis is based on that of Kok (1956). The difference is that instead of estimating y by the maximum rate of photosynthesis, an estimation which is not valid for leaves of higher plants because PS II does not limit the maximum rate of photosynthesis until more than about 40% of PS II have been photoinactivated (Lee et al. 1999), we used instead the experimental parameter $1/F_o - 1/F_m$, which in turn was calibrated against the oxygen yield per repetitive single-turnover flash.

References

Anderson JM, Aro E-M (1994) Grana stacking and protection of photosystem II in thylakoid membranes of higher plants under sustained high irradiance: an hypothesis. Photosynth Res 41: 315–326

Anderson JM, Park Y-I, Chow WS (1998) Unifying model for the photoinactivation of photosystem II in vivo under steady-state photosynthesis. Photosynth Res 56: 1–13

Aro E-M, Virgin I, Andersson B (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143: 113–134

Aro E-M, McCaffery S, Anderson JM (1994) Recovery from photoinhibition in peas (*Pisum sativum* L.) acclimated to varying irradiances. Plant Physiol 104: 1033–1041

Barber J (1995) Molecular basis of the vulnerability of photosystem II to damage by light. Aust J Plant Physiol 22: 201–208

Barber J (1998) Photosystem two. Biochim Biophys Acta 1365: 269–277

Baroli I, Melis A (1996) Photoinhibition and repair in *Dunaliella salina* acclimated to different growth irradiances. Planta 198: 640–646

Bell CJ, Rose DA (1981) Light measurement and the terminology of flow. Plant Cell Environ 4: 89–96

Bhaya D, Jagendorf AT (1984) Optimal conditions for translation by thylakoid-bound polysomes from pea chloroplasts. Plant Physiol 75: 832–838

Chow WS (1994) Photoprotection and photoinhibitory damage. Adv Mol Cell Biol 10: 151–196

Chow WS, Hope AB, Anderson JM (1991) Further studies on quantifying photosystem II in vivo by flash-induced oxygen yield from leaf discs. Aust J Plant Physiol 18: 397–410

Havaux M, Strasser RJ, Greppin H (1991) A theoretical and experimental analysis of the qP and qN coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. Photosynth Res 27: 41–55

Hurewitz J, Jagendorf AT (1987) Further characterization of ribosome binding to thylakoid membranes. Plant Physiol 84: 31–34

Hideg E, Kálai T, Hideg K, Vass I (1998) Photoinhibition of photosynthesis in vivo results in singlet oxygen production. Detection via nitroxide-induced fluorescence quenching in broad bean leaves. Biochemistry 37: 11405–11411

Kok B (1956) On the inhibition of photosynthesis by intense light. Biochim Biophys Acta 21: 234–244

Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant 74: 566–574

Jones LW, Kok B (1966) photoinhibition of chloroplast reactions. I. Kinetics and action spectra. Plant Physiol 41: 1037–1043

Lee H-Y, Chow WS, Hong Y-N (1999) Photoinactivation of photosystem II in leaves of *Capsicum annuum*. Physiol Plant 105: 377–384

Melis A (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? Trends Plant Sci 4: 130–135

Miyao M (1994) Involvement of active oxygen species in degradation of the D1 protein under strong illumination ins isolated subcomplexes of photosystem II. Biochemistry 33: 9722–9730

Nagy L, Bálint E, Barber J, Ringler A, Cook KM, Maróti P (1995) Photoinhibition and law of reciprocity in photosynthesis reactions of *Synechocystis* sp. PCC 6803. J Plant Physiol 145: 410–415

Öquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta 186: 450–460

Osmond CB (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants. In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis: from molecular mechanisms to the field. Bios Scientific, Oxford, pp 1–24

Osmond CB, Daley PF, Badger MR, Lüttge U (1998) Chlorophyll fluorescence quenching during photosynthetic induction in leaves of *Abutilon striatum* Dicks. infected with Abutilon mosaic virus, observed with a field-portable imaging system. Bot Acta 111: 390–397

Park Y-I, Chow WS, Anderson JM (1995) Light inactivation of functional photosystem II in leaves of peas grown in moderate light depends on photon exposure. Planta 196: 401–411

Park Y-I, Chow WS, Anderson JM (1997) Antenna size dependency of photoinactivation of photosystem II in light-acclimated pea leaves. Plant Physiol 115: 151–157

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b with four different solvents: verification of the concentration of chlorophyll by atomic absorption spectroscopy. Biochim Biophys Acta 975: 384–394

Prásil O, Adir N, Ohad I (1992) Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. In: Barber J (ed) The photosystems: structure, function and molecular biology. Elsevier, Amsterdam, pp 295–348

Russell AW, Critchley C, Robinson SA, Franklin LA, Seaton GGR, Chow WS, Anderson JM, Osmond CB (1995) Photosystem II regulation and dynamics of the chloroplast D1 protein in *Arabidopsis* leaves during photosynthesis and photoinhibition. Plant Physiol 107: 943–952

Sundby C, McCaffery S, Anderson JM (1993) Turnover of the photosystem II D1 protein in higher plants under photoinhibitory and nonphotoinhibitory irradiance. J Biol Chem 268: 25476–25482

Schweitzer RH, Brudvig GW (1997) Fluorescence quenching by chlorophyll cations in photosystem II. Biochemistry 36: 11351–11359

Thompson LK, Brudvig GW (1988) Cytochrome b-559 may function to protect photosystem II from photoinhibition. Biochemistry 27: 6653–6658

Tyystjärvi E, Aro E-M (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. Proc Natl Acad Sci USA 93: 2213–2218

Tyystjärvi E, Kettunen R, Aro E-M (1994) The rate constant of photoinhibition in vitro is independent of the antenna size of photosystem II but depends on temperature. Biochim Biophys Acta 1186: 177–185

Vasilikiotis C, Melis A (1994) Photosystem II reaction center damage and repair cycle: chloroplast acclimation strategy to irradiance stress. Proc Natl Acad Sci USA 91: 7222–7226

Walters RG, Horton P (1993) Theoretical assessment of alternative mechanisms for non-photochemical quenching of PSII fluorescence in barley leaves. Photosynth Res 36: 119–139