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Photoinactivation of Photosystem II by cumulative exposure to short light pulses during the induction period of photosynthesis

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Abstract

Photoinactivation of Photosystem (PS) II in vivo was investigated by cumulative exposure of pea, rice and spinach leaves to light pulses of variable duration from 2 to 100 s, separated by dark intervals of 30 min. During each light pulse, photosynthetic induction occurred to an extent depending on the time of illumination, but steady-state photosynthesis had not been achieved. During photosynthetic induction, it is clearly demonstrated that reciprocity of irradiance and duration of illumination did not hold: hence the same cumulative photon exposure (mol m^{-2}) does not necessarily give the same extent of photoinactivation of PS II. This contrasts with the situation of steady-state photosynthesis where the photoinactivation of PS II exhibited reciprocity of irradiance and duration of illumination (Park et al. (1995) Planta 196: 401–411). We suggest that, for reciprocity to hold between irradiance and duration of illumination, there must be a balance between photochemical (qP) and non-photochemical (NPQ) quenching at all irradiances. The index of susceptibility to light stress, which represents an intrinsic ability of PS II to balance photochemical and non-photochemical quenching, is defined by the quotient (1 – qP)/NPQ. Although constant in steady-state photosynthesis under a wide range of irradiance (Park et al. (1995). Plant Cell Physiol 36: 1163–1169), this index of susceptibility for spinach leaves declined extremely rapidly during photosynthetic induction at a given irradiance, and, at a given cumulative photon exposure, was dependent on irradiance. During photosynthetic induction, only limited photoprotective strategies are developed: while the transthylakoid pH gradient conferred some degree of photoprotection, neither D1 protein turnover nor the xanthophyll cycle was operative. Thus, PS II is more easily photoinactivated during photosynthetic induction, a phenomenon that may have relevance for understorey leaves experiencing infrequent, short sunflecks.

Abbreviations: D1 protein – *psb*A gene product; DTT – dithiothreitol; F_v, F_m, F_o – variable, maximum, and initial (corresponding to open traps) chlorophyll fluorescence yield, respectively; NPQ – non-photochemical quenching; PS – Photosystem; Q_A – primary quinone acceptor of PS II; qP – photochemical quenching coefficient

Introduction

When the photosynthetic apparatus receives excess light, photoinhibition takes place, which is partly due to the inactivation of Photosystem (PS) II (Osmond 1994). In a previous study with pea leaves (Park et al. 1995a, b), we reported that the photoinactivation of PS II in vivo depends on the number of photons absorbed, not the rate of absorption, such that irradiance and duration of illumination give the same extent of PS II inactivation if the photon exposure (mol photons m^{-2}, Bell and Rose 1981) is the same. The reciprocity of irradiance and duration of illumination has been investigated in vitro in relation to the dependence of the loss

of PS II activity on photon exposure in isolated chloroplasts (Jones and Kok 1966). This reciprocity is understandable in in vitro systems where electron acceptors and photoprotective reponses are largely absent. However, it is at first surprising that it also operates in leaves over a wide range of photon exposures (Park et al. 1995a). Recently, we proposed that the reciprocity of irradiance and duration of illumination holds in vivo because, although the excitation pressure on PS II (1 − qP) increases with irradiance, there is a concomitant increase in non-photochemical dissipation, as revealed by an increase in non-photochemical quenching (NPQ), ensuring that the ratio (1 − qP)/NPQ is relatively constant over a wide range of irradiance (Park et al. 1995c). Given that the index of susceptibility of PS II to light stress, (1 − qP)/NPQ, is largely independent of irradiance in leaves during steady-state photosynthesis, it is the total absorbed photons, rather than the rate of absorption, that predominantly determines the extent of photoinactivation of PS II.

To investigate further this phenomenon of reciprocity of irradiance and duration of illumination in relation to PS II photoinactivation, we were eager to test it under a wider range of conditions, and in other plant species. Our previous demonstration of reciprocity in pea leaves was conducted under conditions of steady-state photosynthesis. In this study, we turned our attention to the induction period of photosynthesis following a dark period. Our aim was to study the dependence of photoinactivation of PS II on cumulative photon exposure during the induction period of photosynthesis in leaves of pea, rice and spinach. Our results demonstrate that the reciprocity of irradiance and duration of illumination for the photoinactivation of PS II, as indirectly indicated by chlorophyll fluorescence, does not hold during photosynthetic induction. Further, the index of susceptibility of PS II to light stress is not independent of time or irradiance during photosynthetic induction; this might be the reason for the violation of reciprocity of irradiance and duration of illumination during the photosynthetic induction phase.

Materials and methods

Plant materials

Pea (*Pisum sativum* L. cv Greenfeast) was grown in a growth chamber (12 h light/22 °C; 12 h dark/18 °C) illuminated by fluorescent light (250 μmol photons m^{-2} s^{-1}) as described in Park et al. (1995a). Spinach (*Spinacia oleracea* L. cv. Henderson's hybrid 102) was grown in water culture in a white washed glasshouse under natural light during spring. Rice (*Oryza sativa* L. cv Norin 8) was grown under natural light in a glass cabinet within a phytotron glasshouse (28 °C day/22 °C night).

Inhibitor treatments

Leaf petioles (pea and spinach) and blades (rice) were cut under water and transferred to small Eppendorf tubes containing water (control) or the inhibitors (Sigma, St. Louis, MO, USA): 1 mM DTT (an inhibitor of the xanthophyll cycle, Bilger et al. 1989), 0.6 mM lincomycin (an inhibitor of chloroplast-encoded protein synthesis) or 1 μM nigericin (an uncoupler). They were allowed to take up water or an inhibitor for 2 h in a gentle air stream under dim light (20 μmol photons m^{-2} s^{-1}). The bulk concentrations of inhibitors in leaf tissues, estimated according to Bilger and Björkman (1994), were 1.5 mM DTT, 1.2 mM lincomycin and 1.5 μM nigericin.

Light treatments

Two types of light treatments were used. (i) To investigate changes of chlorophyll fluorescence parameters as a function of cumulative photon exposure in control or inhibitor-treated leaves, the upper surface of each of the leaf pieces was exposed to the exciting light in a portable fluorometer (Plant Efficiency Analyzer, Hansatech, UK) with various durations of illumination: its light source consists of six light-emitting diodes which provide red light (up to 3600 μmol photons m^{-2} s^{-1} with a peak wavelength at 650 nm), focused onto a small spot of a leaf defined by the aperture of a leaf clip. We used the light source both as excitation light for the chlorophyll fluorescence measurement and as a strong light source to induce photoinactivation of PS II during the induction phase of photosynthesis. (ii) To induce photoinactivation of PS II for measurement of effects on the number of functional PS II reaction centres, leaf discs of pea were exposed to white light (3600 μmol photons m^{-2} s^{-1}) from a projector lamp; the beam of white light was broad enough to illuminate an entire leaf disc.

Measurements of photosynthesis, functional PS II complexes and time-dependent changes in chlorophyll fluorescence yield

Measurement of photosynthetic oxygen evolution in steady light (1% CO_2 in air) was made using a leaf-disc oxygen electrode (Hansatech, UK) thermostatted at 25 °C. The same electrode also served for the determination of functional PS II reaction centres in vivo using saturating single-turnover flashes (Chow et al. 1991). Measurement of the time-course of changes in chlorophyll fluorescence yield of leaf discs during illumination in air was conducted using the PAM pulse-modulated fluorescence measuring system (Walz, Germany). To record time-dependent changes in chlorophyll fluorescence quenching parameters during photosynthetic induction, a spinach leaf attached to the plant was first dark-treated for 30 min. Actinic light (either 240 or 500 μmol photons m^{-2} s^{-1}) was turned on at time t = 0. At time t = 2 s, a saturating pulse (8000 μmol photons m^{-2} s^{-1}, duration 0.7 s) was applied, and again at 5-s intervals thereafter. Thus, the time-course of changes in photochemical quenching (qP, van Kooten and Snel (1990)), non-photochemical quenching (NPQ = $F_m/F'_m - 1$, where F_m and F'_m are maximum fluorescence yield after dark treatment and during illumination, respectively) and the suceptibility index of PS II to light stress, (1 − qP)/NPQ, could be calculated as a function of induction time.

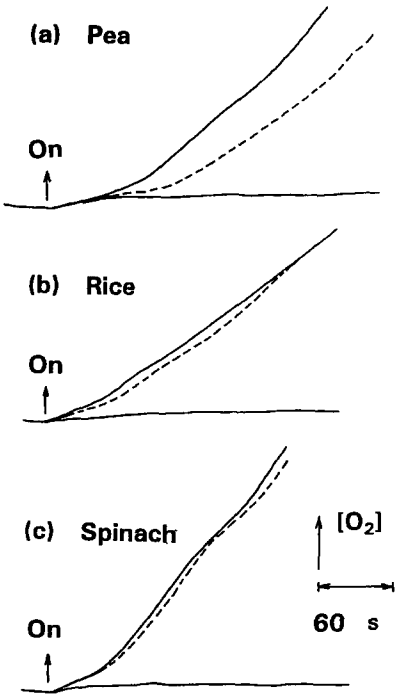

Fig. 1. O_2 evolution during the induction period of photosynthesis of pea (a), rice (b) and spinach (c) leaf discs. The broken trace for each leaf was obtained by illumination with light from a projector (804 μmol photons m^{-2} s^{-1}) after 30 min dark, while the solid traces refer to re-illumination after a further 30 min dark treatment. The near-horizontal traces represent a heating effect of the actinic light, recorded by substituting a leaf disc with a disc of black cloth.

Results

The time-course of oxygen evolution during photosynthetic induction

Leaves of pea, rice and spinach all showed typical curves of photosynthetic induction: the rate of photosynthesis rose gradually, and became steady only after about 3 min of illumination at 804 μmol photons m^{-2} s^{-1} in air containing 1.1% CO_2 (Fig. 1, broken trace for each species). When photosynthesis was measured again after a further dark interval of 30 min, similar photosynthetic induction curves were obtained (Fig. 1, solid trace for each species). In particular, the rates of photosynthesis in the beginning of the induction period were very low for all the species. From these results, it may be anticipated that if a dark-treated leaf disc is illuminated with strong light for a short time after dark intervals of 30 min, and the cycle of dark period/light pulse is repeated to give a cumulative photon exposure, it will experience photoinactivation of PS II under conditions where limited photosynthesis or photoprotective responses have developed.

Photoinactivation of PS II caused by strong light at the beginning of the induction period

To probe the photoinactivation of PS II at the beginning of the photosynthetic induction period, chlorophyll fluorescence parameters of leaf discs from each species were measured repeatedly with dark intervals of 30 min between excitation pulses of strong light. Excitation light from red light-emitting diodes was used with varying duration; the cumulative photon exposure (mol photons m^{-2}, Bell and Rose 1981) given to a leaf was calculated as the product of irradiance and duration of each light pulse, summed over the number of light pulses given. The photoinactivation of PS II resulting from the repeated pulse illumination was measured as a decrease of F_v/F_m. When F_v/F_m ratios were plotted against cumulative photon exposure, the curves were close to linear for each duration of excitation pulse;

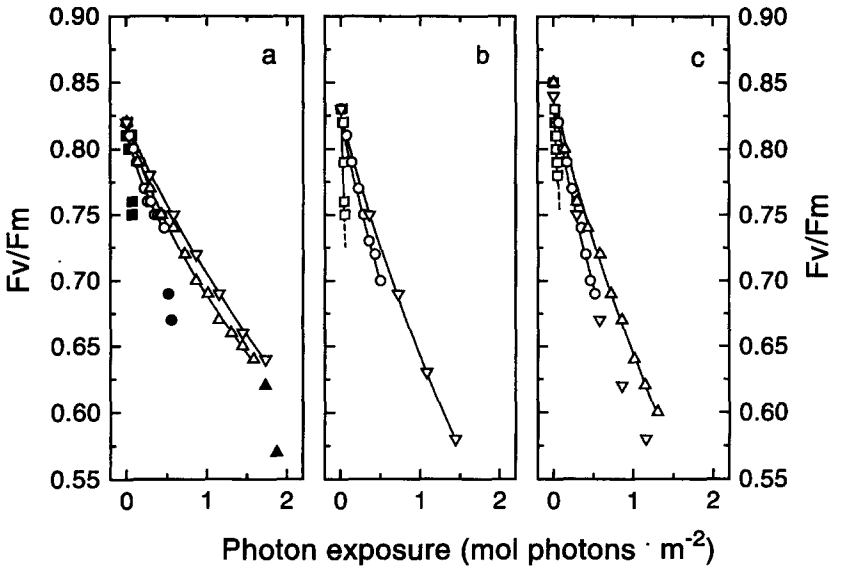

Fig. 2. Changes in F_v/F_m as a function of cumulative photon exposure given to leaves of pea (a), rice (b) and spinach (c). F_v/F_m was measured with the same light pulses (2880 [in (a) and (c)] or 3600 [in (b)] μmol photons m^{-2} s^{-1}, supplied by the red light-emitting diodes of the Plant Efficiency Analyser) as used to induce photoinactivation of PS II; measurements were repeated after 30 min-dark intervals, using light pulses of durations: 2 (□), 15 [○in (a)], 20 [○in (b) and (c)], 50 (Δ) or 100 (▽) seconds. Solid symbols refer to measurements made after an overnight dark period.

this was the case for all plants tested (Fig. 2). However, the slopes of the curves for different pulse durations were different: the shorter the duration of the excitation pulse, the steeper was the slope.

F_o, the fluorescence yield corresponding to open PS II reaction centres, is a parameter whose rise indicates PS II reaction centre malfunction (Franklin et al. 1992; Park et al. 1995a,b). It is seen that F_o increased with increase in cumulative photon exposure given to leaves of pea, rice and spinach (Fig. 3). However, the different species behaved somewhat differently: the F_o of rice rose more sharply when the duration of the excitation pulse was shorter (Fig. 3b), while the rise of F_o for pea (Fig. 3a) and spinach (Fig. 3c) was less dependent on the duration of the excitation pulses.

To check whether the decrease in F_v/F_m was due to photoinactivation of PS II reaction centres, we also measured the number of functional PS II reaction centres. After illumination of leaf discs repeatedly with 20-s pulses of strong white light (3.6 mmol photons m^{-2} s^{-1}, in a broad-beam that was sufficient to illuminate the entire discs, an effect not achievable with the narrow, focused beam from red light-emitting diodes of the Plant Efficiency Analyzer), the decrease in functional PS II reaction centres was determined in a leaf disc oxygen electrode using single-turnover flashes (Chow et al. 1991). The results showed that a photon exposure of 0.65 mol m^{-2} decreased the number of functional PS II reaction centres to about 80% of the control value, while F_v/F_m decreased from 0.82 to 0.68.

Effects of inhibitors on PS II photoinactivation by illumination with strong light in the induction period of photosynthesis of pea leaves

To probe the role of various photoprotective mechanisms during the induction of photosynthesis, we used several inhibitors: the uncoupler, nigericin, inhibits the formation of the transthylakoid pH gradient (Ögren 1991); lincomycin is an inhibitor of chloroplast-encoded protein synthesis; and DTT inhibits the conversion of violaxanthin to zeaxanthin (Bilger et al. 1989) which helps in the dissipation of excess light as heat (Demmig-Adams and Adams 1992). The inhibitors were taken up through the petioles or blades of cut leaves placed in room light for two hours prior to light treatment (Park et al. 1995a,b). After placing the leaves in darkness for 30 min, measurements were made of their chlorophyll fluorescence parameters using 15-s pulses of strong light (2.9 or 3.6 mmol photons m^{-2} s^{-1}, supplied by the red light-emitting diodes of the Plant Efficiency Analyzer, Hansatech), which also served to bring about photoinactivation

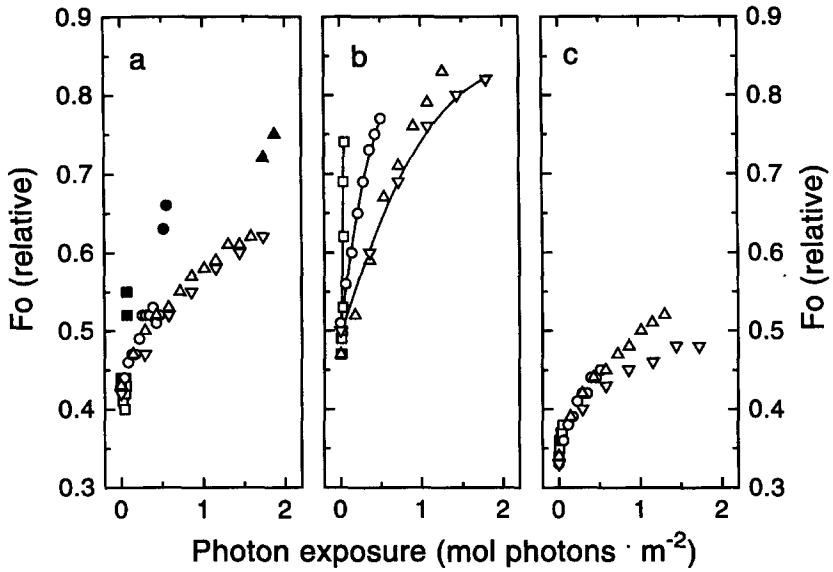

Fig. 3. The increase of F_0 (an indicator of PS II reaction centre malfunction) as a function of cumulative photon exposure given to leaves of pea (a), rice (b) and spinach (c). Measurements and symbols as for Fig. 3.

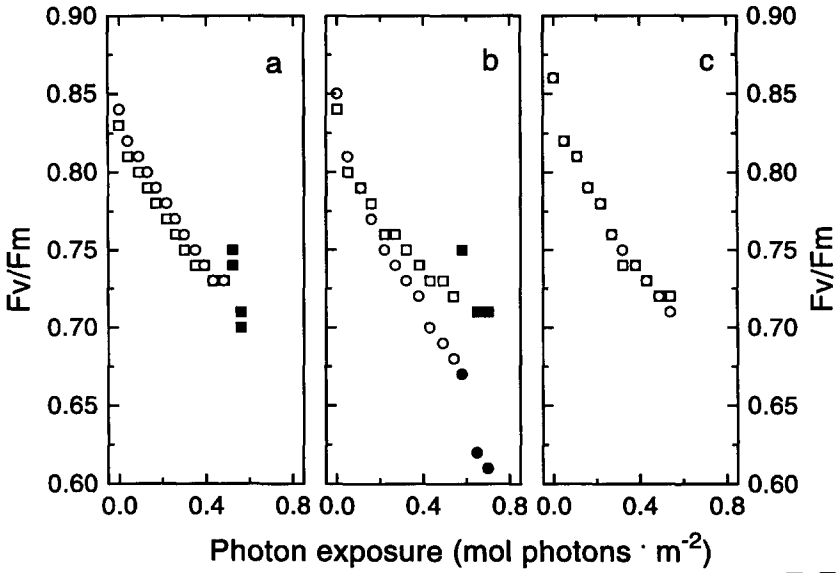

Fig. 4. The decline of F_v/F_m in pea leaf discs as a function of cumulative photon exposure given in the absence (□, ■) or presence (○, ●) of prior uptake of the inhibitors, lincomycin (a), nigericin (b) or DTT (c). Light pulses of duration 15 s at an irradiance of 2880 (a) or 3600 (b and c) μmol photons m^{-2} s^{-1} were given, separated by dark intervals of 30 min. Solid symbols refer to measurements made after an overnight dark treatment.

of PS II during the photosynthetic induction period, as described in Section 3. The results show that, in comparison with the control, only nigericin (Fig. 4b) enhanced photoinactivation of PS II, while lincomycin and DTT had little or no effect (Fig. 4a and c).

Fluorescence quenching parameters and the index of susceptibility of PS II to light stress, (1 – qP)/NPQ, during photosynthetic induction

During the early phase of photosynthetic induction, utilization of reducing power is not yet maximized. Therefore, Q_A in PS II was predominantly reduced initially, becoming more oxidized as photosynthetic induction

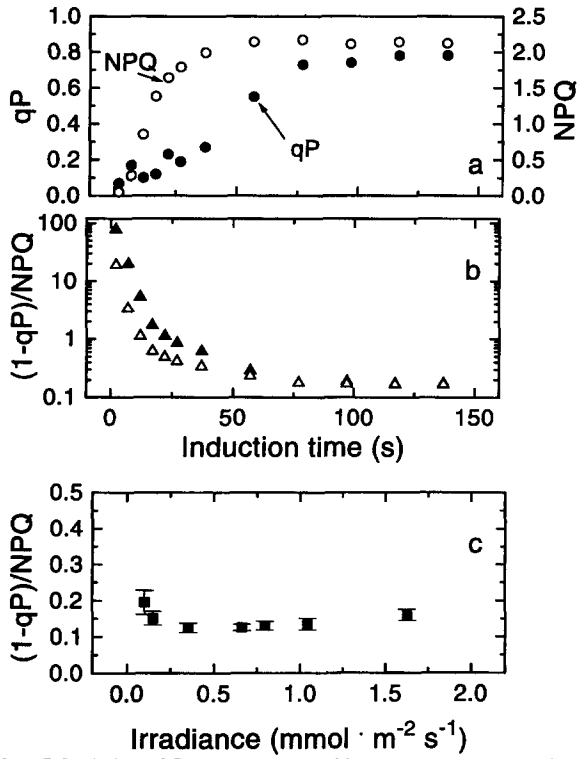

Fig. 5. Variation of fluorescence quenching parameters during photosynthetic induction. (a) The increase in qP (●) and NPQ (○) during photosynthetic induction in a spinach leaf attached to the plant and exposed to 500 μmol photons m^{-2} s^{-1}, supplied as white light from a projector. The leaf was dark-adapted for 30 min before each measurement. (b) Rapid decline of (1 − qP)/NPQ, an index of susceptibility of PS II to light stress, as a function of time during induction of photosynthesis. Actinic irradiance was 240 (▲) or 500 (△) μmol photons m^{-2} s^{-1}. (c) The (1 − qP)/NPQ ratio in spinach leaves undergoing steady-state photosynthesis in various irradiances. Values are means ± S.E. for 4 leaves.

progressed. The photochemical quenching parameter, qP, increased rapidly in spinach during increasing photosynthetic induction (closed circles, Fig. 5a). Similarly, non-photochemical quenching (NPQ) was initially very low, but increased to a peak in about 1 min (open circles, Fig. 5a). The reduction state of Q_A in PS II, given by the chlorophyll fluorescence parameter (1 − qP), is a determining factor in photoinactivation of PS II (Ögren 1991; Öquist et al. 1992). On the other hand, protection against photoinactivation of PS II can occur via light-harvesting antenna dissipation of excitation energy, associated directly or indirectly with the transthylakoid pH gradient, conveniently indicated by NPQ, a coefficient of non-photochemical quenching of chlorophyll fluorescence (Bilger and Björkman 1990). A useful parameter to assess the contribution of photochemical and non-photochemical quenching of chlorophyll fluorescence in relation to the susceptibility of PS II to light stress is the ratio (1 − qP)/NPQ, which is a measure of 'excess photons' or 'light stress' (Osmond 1994). Figure 5b demonstrates that the index of susceptibility of PS II to light stress in spinach leaves was extremely high at the beginning of illumination, but declined to a steady value of about 0.17 by 100 s. Significantly, a doubling of actinic irradiance led to an approximately 4-fold decrease in the susceptibility index at early times, but to the same steady-state value later. When (1 − qP)/NPQ was plotted against cumulative photon exposure, the curves for the two irradiances were not identical in the first 3 min of the induction period (data not shown). The susceptibility index of spinach leaves previously undergoing steady-state photosynthesis was also determined as a function of irradiance, and found to be largely constant over a wide range of irradiance (Fig. 5c).

Discussion

When the dark-adapted photosynthetic apparatus is exposed to light, a series of regulatory reactions takes place gradually, bringing the whole process of photosynthesis under coordinated control (Walker 1976). In this paper, the dependence of photoinactivation of PS II on cumulative photon exposure during the induction period of photosynthesis in pea, rice and spinach leaves was investigated. To study the photoinactivation of PS II as a function of cumulative photon exposure, we illuminated leaf discs with the strong excitation light from red light-emitting diodes of the Plant Efficiency Analyzer for various durations (from 2 to 100 s) after a 30 min dark interval; the dark interval/light pulse cycle was repeated many times to give various cumulative photon exposures. We used the red light from light-emitting diodes both as exciting light for the emission of fluorescence, and as a light source to induce photoinactivation of PS II, which was monitored by changes in the photochemcial efficiency of PS II, as estimated by the F_v/F_m ratio measured after 30 min dark treatment. We showed that declines in F_v/F_m could be used as an approximate indicator of the loss of functional PS II reaction centres, since, in a separate experiment, functional PS II reaction centres in spinach leaves were decreased to about 80% of the control by a photon exposure of 0.65 mol m^{-2} (provided as 20-s pulses of white light of irradiance 3.6 mmol m^{-2} s^{-1}, in a beam which was broad enough to illuminate entire discs), accompanied by a decrease of F_v/F_m from about 0.82

to 0.68. The results show that measurement of chlorophyll fluorescence is a convenient, qualitative method for studying photoinactivation of PS II in the induction period of photosynthesis. Our previous study with pea leaves demonstrated that although F_v/F_m does not reveal the photoinactivation of a minor population of less-stable PS II, a decline in F_v/F_m (measured after 30 min dark) *is* accompanied by a loss of functional PS II complexes (Park et al. 1995a,b)

Reciprocity of irradiance and duration of illumination does not hold during the induction period of photosynthesis

Photoinactivation of PS II function in vitro (Jones and Kok 1966) and in vivo (Park et al. 1995a,b) obeys a reciprocity law, whereby the extent of photoinactivation of PS II is determined by photon exposure, the product of irradiance and duration of illumination. The initially surprising finding that reciprocity holds for the inactivation of PS II in vivo during steady-state photosynthesis of leaves has been explained by our observation that the steady-state index of susceptibility of PSII to light stress, (1 − qP)/NPQ, is relatively constant over a wide range of irradiance for pea leaves (Park et al. 1995c). Clearly, PS II in vivo has an intrinsic ability to balance photochemical and non-photochemical quenching during steady-state photosynthesis over a wide range of irradiance. This being the case, it means that the extent of photoinactivation of PSII is primarily determined by total photon exposure, and not the rate of photon absorption. In seeking to test this explanation, we investigated photoinactivation of PS II during the induction period of photosynthesis, when rapid changes in photochemical and non-photochemical quenching may lead to a rapidly-changing ratio of (1 − qP)/NPQ, and energy supply is not balanced by energy consumption.

Figure 2 demonstrates that the declines in F_v/F_m with increase in cumulative photon exposure for leaves of pea, rice and spinach were steeper when the duration of each light pulse was shorter; the steep decline in F_v/F_m for short (2 s) pulses was particularly evident in rice and spinach (Fig. 2b and c). Clearly, reciprocity of irradiance and duration of illumination for the photoinactivation of PS II, as indicated by chlorophyll fluorescence, does not hold during the induction period of photosynthesis.

If, as proposed by Park et al. (1995c), reciprocity of irradiance and duration of illumination in vivo depends on (1 − qP)/NPQ being largely constant, despite changing irradiance or duration of illumination in steady-state photosynthesis, then one expects that a direct measurement of this parameter during the photosynthetic induction period would reveal whether the violation of reciprocity is related to a non-constant index of susceptibility to light stress. Figure 5b shows that, indeed, the susceptibility index declined rapidly by three orders of magnitude during the onset of illumination of a spinach leaf at a given irradiance, eventually settling down to a steady-state ratio of about 0.17 for spinach, a value close to the steady-state value of pea leaves acclimated to moderate or high growth irradiance (Park et al. 1995c), and of spinach leaves previously undergoing steady-state photosynthesis (Fig. 5c). However, in the initial period before steady-state photosynthesis was reached, the index of susceptiblity of PS II to light stress was very high, suggesting that PS II was extremely vulnerable to light stress in the early phase of photosynthetic induction (Fig. 5b): that is, short cumulative light pulses of a given irradiance would be much more effective in photoinactivation of PS II than a long pulse of the same irradiance and equivalent photon exposure, i.e. the reciprocity law does not hold for photoinactivation of PS II during the induction period of photosynthesis.

Chlorophyll fluorescence yield, F_o, in relation to the induction phase of photosynthesis

The relationship between the decrease of F_v/F_m and increase of cumulative photon exposure showed a similar tendency in the three species examined (Fig. 2). However, the response of F_o (the chlorophyll fluorescence yield corresponding to open PS II traps) to increase of cumulative photon exposure was peculiar in rice, which showed that the shorter the duration of light pulses, the steeper was the increase of F_o with increase of photon exposure (Fig. 3b), while in the other two species, the difference was less obvious (Fig. 3a and c). An increase of F_o after a light treatment is indicative of malfunction of the PS II reaction centre (Franklin et al. 1992; Park et al. 1995a); our observed increase in F_o following the application of cumulative light pulses (Fig. 3) is consistent with the decline in F_v/F_m (Fig. 2) being indicative of photoinactivation of PS II under our experimental conditions.

Limited operation of photoprotective strategies during photosynthetic induction

The extremely high values of (1 – qP)/NPQ during the induction period of photosynthesis for spinach leaves are probably mainly due to extensive reduction of Q_A [high (1 – qP)] when utilization of reducing power was not yet maximal. If so, it follows that the protective effect of oxygen, as a likely in vivo electron acceptor in whole-chain electron transport, might also have been rather limited during the early phase of photosynthetic induction. Indeed, while O_2-dependent quenching of chlorophyll fluorescence has been clearly demonstrated, maximum quenching only occurs after 2 min of illumination of intact chloroplasts (Schreiber and Neubauer 1990).

With regard to other photoprotective strategies, there is an important difference between our present and previous results obtained with lincomycin, which inhibits chloroplast-encoded protein synthesis and hence D1 protein turnover. Previously, when photoinactivation of PS II was studied under steady-state conditions, it was demonstrated, by the large effect of lincomycin on the loss of functional PS II even under low photon exposure, that de novo synthesis of the D1 protein represents one of the most important photoprotective strategies (Park et al. 1995a,b). In contrast, the present study demonstrates that lincomycin had no noticeable effect on photoinactivation of PS II during the induction phase of photosynthesis (Fig. 4a). The observed lack of inhibition of D1 protein synthesis during photosynthetic induction is reasonable, considering that the development of the repair process is stimulated by light, and that the very short period of photosynthesis might not have been enough for significant D1 protein turnover to occur. It may be necessary for the stromal pH to be maintained at an optimum value over a much longer period than 100 s (the longest period of photosynthetic induction used here) before sufficient amounts of D1 protein can be synthesized (Chow 1994). Further, light modulates the translation of the *psb*A mRNA by changing the redox state of thioredoxin via reduced ferredoxin (Danon and Mayfield 1994); during photosynthetic induction, sufficient time may not have lapsed to allow adequate reducing power to develop for the ensuing translation of *psb*A mRNA.

The relative importance of the photoprotective mechanism of the xanthophyll cycle in limiting the photoinactivation of PSII can be determined by using DTT-treated leaves, since DTT inhibits the de-epoxidation of violaxanthin to zeaxanthin (Bilger and Björkman 1990). However, DTT had no effect on the photochemical efficiency of PS II (F_v/F_m) during the induction period (Fig. 4c), in contrast to DTT-treated pea leaves undergoing steady-state photosynthesis (Park et al. 1995a). During steady-state photosynthesis, DTT only became effective above a threshold photon exposure of 2 mol m^{-2}(Park et al. 1995a). As this threshold photon exposure is very considerably higher than was established during the cumulative photon exposure needed for the induction of photosynthesis (Fig. 2), it was to be expected that the xanthophyll cycle would be inoperative during photosynthetic induction, as demonstrated here (Fig. 4c).

However, the uncoupler, nigericin, had some effect on the photoinactivation of PS II during the induction of photosynthesis (Fig. 4b). This also fits in with our previous study of steady-state photosynthesis: the transthylakoid pH gradient (abolished by nigericin) was immediately engaged in pea leaves at very low photon exposure and thereafter increased asymptotically with increasing photon exposure (Park et al. 1995a). Hence, we expected that an uncoupler would dissipate the transthylakoid pH gradient, even during the induction period of photosynthesis, as indeed was the case (Fig. 4b).

Concluding remarks

During the induction period of photosynthesis, the photoinactivation of PS II, as indicated by chlorophyll fluorescence measurements, does not obey the reciprocity law, so that irradiance and duration of illumination are not equivalent at the same photon exposure. This contrasts with the situation of steady-state photosynthesis, where the photoinactivation of PS II obeys the reciprocity law and depends only on the total photon absorption, and not the rate of absorption (Park et al. 1995a). We suggest that reciprocity of irradiance and duration of illumination seems to depend on a constant index of susceptibility of PS II to light stress, (1 – qP)/NPQ, at varying irradiance, a condition that is satisfied in the steady-state photosynthesis but not during the photosynthetic induction period.

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