Plant Physiol. (1995) 107: 943–952

Photosystem II Regulation and Dynamics of the Chloroplast D1 Protein in *Arabidopsis* Leaves during Photosynthesis and Photoinhibition[1]

A. Wendy Russell, Christa Critchley*, Sharon A. Robinson, Linda A. Franklin, George G. R. Seaton, Wah Soon Chow, Jan M. Anderson, and C. Barry Osmond

Department of Botany, The University of Queensland, Queensland 4072, Australia (A.W.R., C.C.); Research School of Biological Sciences and Cooperative Research Centre for Plant Science, Institute of Advanced Studies, The Australian National University, GPO Box 475, Canberra, Australian Capital Territory 2601, Australia (S.A.R., L.A.F., G.G.R.S., C.B.O.); and Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, GPO Box 1600, Canberra, Australian Capital Territory 2601, Australia (W.S.C., J.M.A.)

Arabidopsis thaliana **leaves were examined in short-term (1 h) and long-term (10 h) irradiance experiments involving growth, saturating and excess light. Changes in photosynthetic and chlorophyll fluorescence parameters and in populations of functional photosystem II (PSII) centers were independently measured. Xanthophyll pigments, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-binding sites, the amounts of D1 protein, and the rates of D1 protein synthesis were determined. These comprehensive studies revealed that under growth or light-saturating conditions, photosynthetic parameters remained largely unaltered. Photoprotection occurred at light saturation indicated by a dark-reversible increase in nonphotochemical quenching accompanied by a 5-fold increase in antheraxanthin and zeaxanthin. No consistent change in the concentrations of functional PSII centers, DCMU-binding sites, or D1 protein pool size occurred. D1 protein synthesis was rapid. In excess irradiance, quantum yield of O_2 evolution and the efficiency of PSII were reduced, associated with a 15- to 20-fold increase in antheraxanthin and zeaxanthin and a sustained increase in nonphotochemical quenching. A decrease in functional PSII center concentration occurred, followed by a decline in the concentration of D1 protein; the latter, however, was not matched by a decrease in DCMU-binding sites. In the most extreme treatments, DCMU-binding site concentration remained 2 times greater than the concentration of D1 protein recognized by antibodies. D1 protein synthesis rates remained unaltered at excess irradiances.**

During the last decade studies of photosynthesis and photoinhibition in higher plants have been driven by many advances in our understanding and by the availability of new techniques for in vivo and in vitro investigations. Following the biochemical studies of Kyle et al. (1984) with photoheterotrophic *Chlamydomonas*, attention has focused on a central role for the synthesis and degradation of the D1 protein of the heterodimer of the PSII reaction center (Critchley, 1988; Barber and Andersson, 1992; Critchley et al., 1992; Prásil et al., 1992; Sundby et al., 1992, 1993; Aro et al., 1993b). At the same time biophysical and physiological studies have exploited Chl fluorescence analysis to interpret PSII function in vivo (Bilger and Schreiber, 1986; Baker and Horton, 1987; Björkman, 1987; Weis and Berry, 1987; Krause, 1988; Krause and Weis, 1991). The recognition of a potential role for the xanthophyll cycle in the dissipation of excess photons (Demmig et al., 1987) has led to increased attention to the structure and function of the light-harvesting antennae of PSII and their relationships to reaction center function under prolonged exposure to photon fluxes in excess of those required to saturate photosynthesis (Horton et al., 1991; Demmig-Adams and Adams, 1992; Ruban et al., 1993).

As was pointed out recently (Osmond, 1994), each of the above approaches has developed its own culture of research and, to an extent, its own set of interpretations independent of the other. In spite of some attempts to undertake comprehensive experiments (Bradbury and Baker, 1986; Chow et al., 1989; Öquist et al., 1992; Aro et al., 1993a), until now they have fallen short in one way or another. It is now recognized, for example, that the D1 protein turnover cycle must be interpreted against a background of the populations of down-regulated PSII centers with different functional attributes (Giersch and Krause, 1991; Walters and Horton, 1993; Long et al., 1994; Osmond, 1994).

The research reported here attempts to approach more closely the desirable objective of a truly comprehensive analysis of relationships between photosynthesis and photoinhibition in *Arabidopsis*, an organism long favored for genetic manipulation and long selected for growth under poorly lit and poorly controlled laboratory conditions. Little is known about the photosynthetic properties of laboratory strains of *Arabidopsis* in anything approaching nat-

[1] This research was supported by a grant from the Cooperative Research Centre for Plant Science and by a University of Queensland Enabling Grant, both to C.C.

* Corresponding author; e-mail c.critchley@botany.uq.oz.au; fax 61–7–365–1699.

Abbreviations: D1, D1 reaction center protein of PSII; F_m, F_o, and F_v, maximal, initial or intrinsic, and variable Chl fluorescence, respectively; Q_A, primary electron accepting plastoquinone of PSII; q_N and q_P, coefficients of nonphotochemical and photochemical Chl fluorescence quenching, respectively.

ural environments. Before embarking on investigations into the genetic manipulation of PSII structure and function in this organism, it was deemed prudent to understand and integrate these processes in the wild-type parents as a point of reference.

We have grown plants under optimal controlled conditions of relatively low light intensity and then studied a range of photosynthetic parameters under growth irradiance, irradiance that saturates photosynthesis, and two irradiances exceeding saturation (approaching 50 and 100% of full sunlight) that result in significant photoinhibition. Comprehensive measurements of photosynthetic light-response curves, Chl fluorescence and fluorescence-quenching parameters, PSII function in vivo, and xanthophyll pigment composition have been made as a background to changes in D1 protein synthesis, pool size, and properties in each treatment.

MATERIALS AND METHODS

Plant Growth Conditions

Arabidopsis thaliana (cv Bensheim) seed was planted in compost in 15-cm-deep pots and covered with plastic wrap for 4 d of germination. Pots were then uncovered and young plants exposed to 200 to 230 μmol m^{-2} s^{-1} from a combination of fluorescent and incandescent sources in a 12-h day-night cycle with 22°C by day and 17°C at night. Plants were irrigated with Hoagland solution in the morning and with water each afternoon. Leaves (2–3 cm^2) were sampled from plants in the 4 to 6 weeks after planting but before flowering, which occurred after 6 weeks.

Experimental Irradiance Treatments

Selected rosette leaves of similar size and position (similar age) were severed under water at the petiole and floated, upperside up with petiole submerged, on distilled water in metal or plastic trays. Trays were placed on a gently shaking platform in a temperature-controlled (20°C) water bath under a Philips HPLR 1000-W mercury vapor lamp so that the movement also averaged the small variations in the light environment. All leaves were initially exposed to 220 to 250 μmol m^{-2} s^{-1} (growth light) to adjust for 1 h. This irradiance was achieved by using layers of shade cloth between the trays in addition to a glass heat filter (Schott 115 Tempax), which also filtered out UV wavelengths. Subsequently, either leaves continued to be exposed at growth irradiance or some sheets of shade cloth were removed to expose leaves to either 420 (saturating light), 1350, or 2200 to 2300 μmol m^{-2} s^{-1} (irradiance in excess of saturation) for up to 10 h. Concurrent measurements of photosynthetic O_2 evolution, fluorescence parameters, fluorescence quenching, O_2 flash yield, [^{14}C]DCMU-binding site concentration, and D1 protein labeling were made on the same batches of leaves that had been collected in the early morning. All experiments were replicated on separate days.

Chl Fluorescence Quenching and Photosynthetic Oxygen Evolution

Fluorescence parameters (F_v/F_m and F_o, dark adapted) were measured on four leaves selected at frequent intervals after transfer to treatments. Leaves were dark adapted for 10 min in the leaf clip of a Hansatech Plant Efficiency Analyser (Hansatech Ltd., King's Lynn, Norfolk, UK), operated at 80% maximum excitation light (1870 μmol m^{-2} s^{-1}) for 5 s. Other leaves were sampled before and at intervals during treatments at growth irradiance, saturating light, or excess irradiance for the simultaneous measurement of photosynthetic O_2 evolution and modulated Chl fluorescence (Seaton and Walker, 1992). The rate of O_2 evolution of illuminated 1.5-cm^2 leaf discs, in saturating CO_2, was measured in a leaf-disc electrode chamber (model LD2; Hansatech) maintained at 20°C. Modulated Chl fluorescence was simultaneously recorded using a PAM 101 Chlorophyll Fluorometer/FL 103 additionally equipped with PAM 102/102-FR, PAM 103/Schott KL1500 electronic flash lamp, and polyfurcated fiberoptic (H. Walz, Effeltrich, Germany). At the end of the dark-adaptation period (20–30 min), respiration rate and F_o were recorded, followed by a 1-s saturating flash to measure F_m. (F_m values from dark-adapted control leaves measured prior to any experimental light treatments were used for determinations of q_N). An ascending series of actinic irradiances was then programmed so that O_2 evolution rate and F_s were measured at steady state immediately followed by a saturating flash to detect $F_{m'}$ followed by a short "dark" period with weak far-red light (3 W m^{-2}, 735 nm) to ensure Q_A oxidation during F_o measurement. Calculations of quenching coefficients were made according to the method of Bilger and Schreiber (1986) and Genty et al. (1989). Saturating flashes were triggered, and signals were recorded and analyzed with an IBM-compatible 386 computer equipped with an analog-to-digital conversion board (model IF-1, Hansatech).

Functional PSII-O_2 Flash Yield

The number of functional PSII reaction centers capable of O_2 evolution in leaves was determined from the O_2 yield per single-turnover flash during repetitive (10 Hz) flash illumination and expressed on a Chl basis (Chow et al., 1991). The duration of the flash, measured as the full width at half-peak intensity, was approximately 2.5 s. A small heating artifact due to the flashes was taken into account. Background far-red light was used to avoid any limitation of electron transport by PSI.

Carotenoid Pigment Determinations

Leaf carotenoid pigments, particularly the xanthophyll cycle pigments violaxanthin, antheraxanthin, and zeaxanthin, were measured in samples taken before and during the light treatments. Two discs (0.75 cm^2) were punched from leaves and stored in liquid nitrogen. Samples were ground in liquid nitrogen and extracted in 100% acetone (acetone-water mixtures were not as effective in extraction). Pigments were measured using the HPLC method

and solvents A and B described by Gilmore and Yamamoto (1991). The pigments were separated on a Spherisorb ODS1 column (Alltech Associates, Sydney, Australia) at a flow rate of 1.5 mL min^{-1} for solvent A and 1 mL min^{-1} for solvent B and with a 20-μL injection volume. Pigment concentrations were calculated using standards as described earlier (Robinson et al., 1993).

Measurements of D1 Protein Synthesis and Pool Size

Leaves were labeled routinely by floating them in culture tray wells with petioles submerged in a solution of 50 Ci (1.85×10^6 Bq) of carrier-free L-[^{35}S]Met in 3 mL of distilled water (13 nM). Labeling began 60 min prior to, and continued throughout, the experimental treatments. Samples of two leaves chosen at random were taken 30 min before and then at intervals during the experimental treatments and were immediately frozen in liquid nitrogen. Preliminary experiments with leaf discs established that the rates of total L-[^{35}S]Met uptake were similar in carrier-free treatments and in treatments containing 0.1 mM Met. Uptake was linear for 8 h, but autoradiography of the discs using a Phosphor Imager (model 400 S, Molecular Dynamics, Sunnyvale, CA) showed that the label was much greater at the edges of discs, and little label reached the center of 9-mm diameter discs. Autoradiography of L-[^{35}S]-Met uptake into intact leaves showed that, although label was concentrated in the vascular tissues, this mode of delivery ensured label transfer to all cells in the tissue.

The frozen leaves were processed on the day of sampling by grinding them with a mortar and pestle in ice-cold isolation buffer (0.33 M sorbitol, 25 mM Tricine/KOH at pH 7.8, 5 mM $MgCl_2$, 10 mM NaCl), and thylakoid membranes were spun down in an Eppendorf centrifuge at 14,000 rpm for 7 min. The pellet was washed once (in isolation buffer lacking osmoticum) and resuspended in 100 μL of isolation buffer, which yielded 0.5 to 1 mg Chl mL^{-1}. Chl was determined by extraction from a 5-μL aliquot in 80% buffered acetone and measured in a Hitachi U-3200 spectrophotometer (Porra et al., 1989). The thylakoid isolation procedure was carried out at 4°C.

Samples were suspended in solubilization buffer (0.1 M Tris-HCl, pH 8.8, 5% glycerol, 3% lithium dodecyl sulfate, 0.3% DTT), loaded on an equal Chl basis on a lithium dodecyl sulfate/15 to 23% polyacrylamide/4 M urea gradient gel, and run overnight at 12 mA and 7°C. Gels were dried following treatment with an enhancer containing 2,5-diphenyloxazole in methylnaphthalene. The signal from labeled proteins was analyzed using the Phosphor Imager. The gels were also autoradiographed and analyzed by densitometry using a GS300 scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA). Gels were also blotted onto nitrocellulose and allowed to react with an antibody specific to the D1 protein. The immune reaction was visualized using Enhanced ChemiLuminescence (Amersham, UK) and quantified with the scanning densitometer.

Total D1 Protein Content: [^{14}C]DCMU Binding

Leaf samples were taken and thylakoids isolated as described above. The total D1 protein content was then estimated by measuring the specific binding of [^{14}C]DCMU to the D1 protein. [^{14}C]DCMU was added to suspensions of thylakoids (50 μM Chl) to give concentrations ranging from 80 to 300 nM. After dark equilibration of 3 min, the suspensions were centrifuged in an Eppendorf microfuge for 3 min. The difference between the herbicide added to a thylakoid suspension and the amount remaining in the supernatant was taken as the amount of bound DCMU. The number of DCMU-binding sites was determined by linear regression in a double-reciprocal plot (Tischer and Strotmann, 1977; Chow et al., 1990).

RESULTS

Short-Term Experiments

Exposure of *Arabidopsis* leaves from plants grown at 220 μmol photons m^{-2} s^{-1} to saturating irradiance (420 μmol photons m^{-2} s^{-1}) for 1 h had no effect on dark-adapted F_v/F_m or F_o (Fig. 1A), indicating no sustained effect on

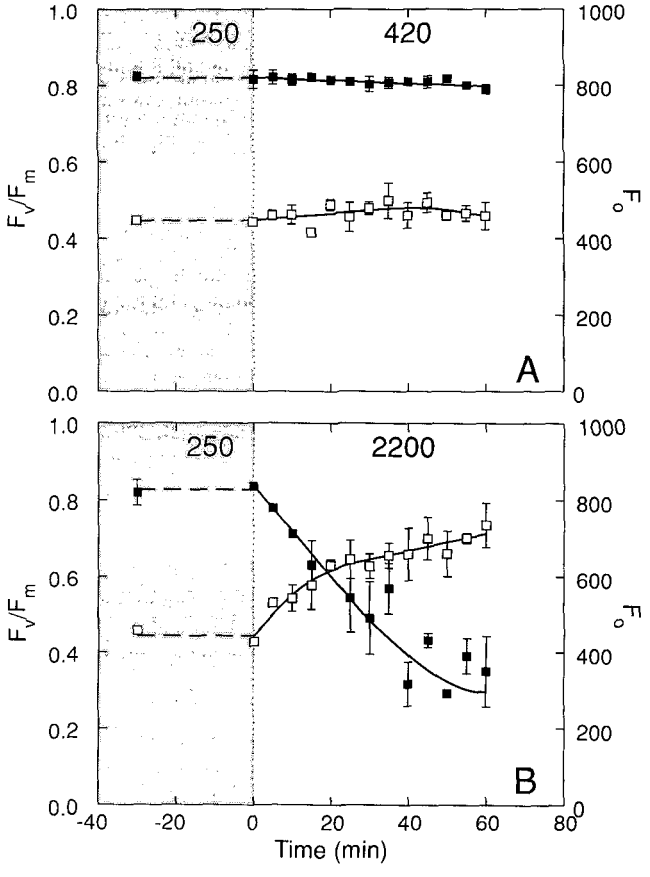

Figure 1. Dark-adapted (10 min) fluorescence parameters F_o (□) and F_v/F_m (■) measured in *Arabidopsis* leaves as a function of time after exposure to growth irradiance (250 μmol photons m^{-2} s^{-1}; shaded area) and after transfer to saturating (A) or excess irradiance (B) (420 or 2200 μmol photons m^{-2} s^{-1}, respectively).

photosynthetic efficiency. However, treatment at 10× growth irradiance caused substantial photoinhibition during this period, as indicated by the decline in dark-adapted F_v/F_m and elevation of F_o (Fig. 1B). These results were confirmed by the photosynthetic and fluorescence-quenching analyses displayed in Figure 2. After 1 h of treatment at saturating irradiance there was no significant change in the photosynthetic light-response curve (Fig. 2A), the light dependence of the change in PSII efficiency (Fig. 2B), or the light dependence of the changes in q_N and q_P (Fig. 2, C and D). However, exposure to 2200 μmol photons m^{-2} s^{-1} led to a large decrease in the quantum yield of O_2 evolution (Fig. 2E) and the efficiency of PSII (Fig. 2F) within 20 min, presumably related to the increase in q_N (Fig. 2G). These changes were even more pronounced after 1 h (Fig. 2, E–G). It is particularly interesting that the maximum reduction in PSII efficiency and maximum q_N were engaged at very low light intensity after these photoinhibitory treatments (Fig. 2, F and G). The light dependence of the reduction state of Q_A was not significantly different from controls in any of these experiments (Fig. 2, D and H).

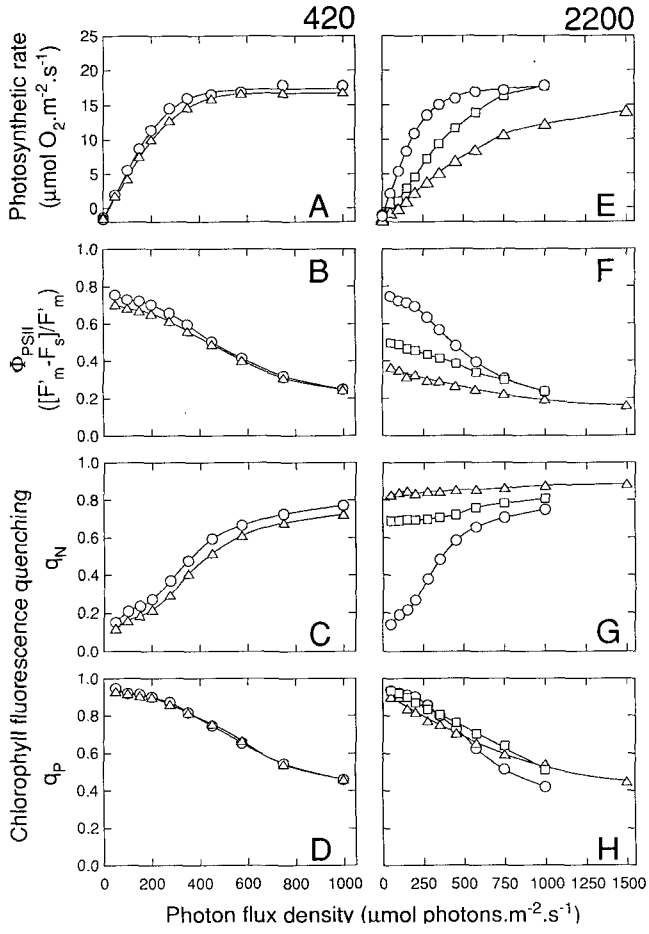

Figure 2. Light-response curves for CO_2-saturated photosynthesis (A and E), PSII efficiency (B and F), q_N (C and G), and q_P (D and H) in *Arabidopsis* leaves. Response functions were measured after exposure to 250 μmol photons m^{-2} s^{-1} for 2 h (○) followed by 25 min (□) or 1 h (△) of treatment at saturating (A–D) or excess irradiance (E–H) (420 or 2200 μmol photons m^{-2} s^{-1}, respectively).

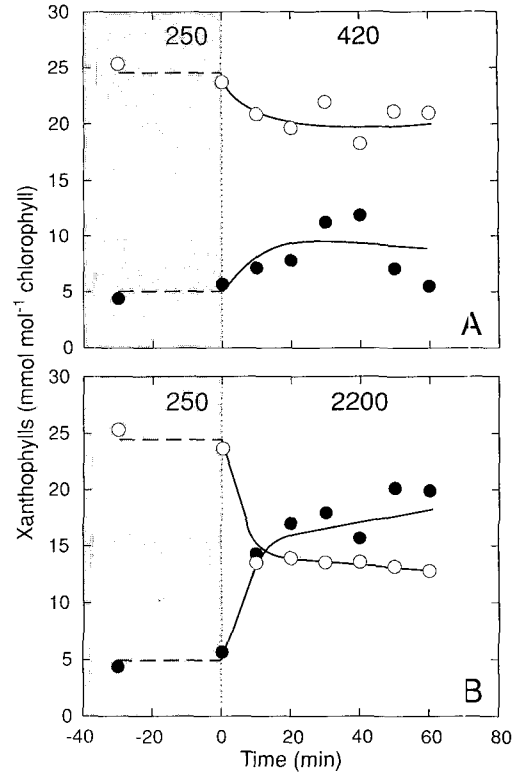

Figure 3. Changes in xanthophyll pigment composition of *Arabidopsis* leaves during exposure to saturating (A) or excess irradiance (B) (420 or 2200 μmol photons m^{-2} s^{-1}, respectively). Conditions were as in Figure 1. ○, Violaxanthin; ●, antheraxanthin plus zeaxanthin.

The changes in xanthophyll pigments in leaves during treatments at 420 and 2200 μmol photons m^{-2} s^{-1} are shown in Figure 3, A and B, respectively. Only about 30% of the total xanthophyll cycle pool was converted to antheraxanthin and zeaxanthin at the lower, saturating irradiance (Fig. 3A), whereas in excess irradiance about 50% of the pool was converted to antheraxanthin and zeaxanthin within 20 min, followed by a slow, further increase during the next 40 min. The sustained increase in zeaxanthin in excess irradiance is presumably related to the sustained and marked change in the light dependence of PSII efficiency and q_N, shown in Figure 2, B and C, versus Figure 2, F and G.

The labeling of D1 protein in *Arabidopsis* leaves with [^{35}S]L-Met was not responsive to irradiance. The inset to Figure 4 shows that the rate of incorporation was similar in the 220 μmol photons m^{-2} s^{-1} pretreatment and after transfer to both 420 and 2200 μmol photons m^{-2} s^{-1}. The total amount of D1 protein, as measured by [^{14}C]DCMU binding, was more or less constant throughout these treatments (Fig. 5A) and there was no change in the $K_{½}$ for DCMU binding (data not shown). Yet, as would be expected from Figures 1 and 2, the concentration of functional PSII centers declined markedly during the 1-h treatment in excess light (Fig. 5B). There were only small changes in both parameters when measured at light saturation.

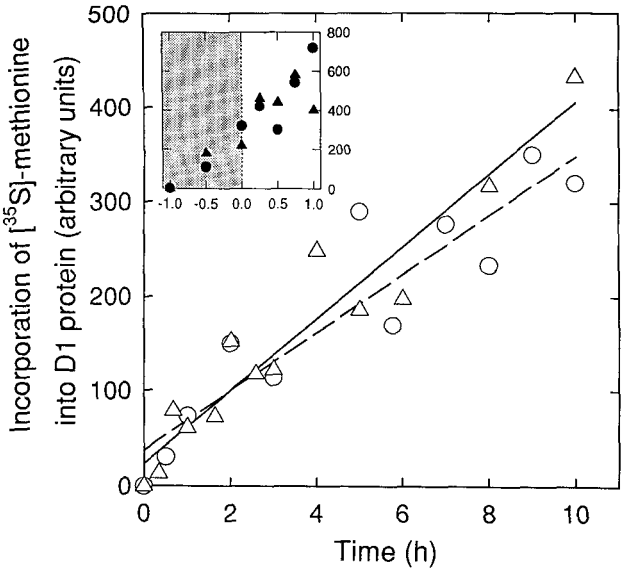

Figure 4. Time course of incorporation of [^{35}S]Met into D1 protein of *Arabidopsis* leaves exposed to saturating or excess irradiance (420 [○, ●] or 2200 [▲, △] μmol m^{-2} s^{-1}, respectively). Inset shows short-term incorporation after 1 h of prelabeling at 220 μmol photons m^{-2} s^{-1} (shaded area) in each case. The rates at the two treatment irradiances are not significantly different (○, $r^2 = 0.85$; $y = 31x + 37.76$; ●, $r^2 = 0.92$; $y = 38.3x + 23.5$).

Long-Term Experiments

The short-term experiments were repeated with an extended range of treatment conditions and for longer periods. Dark-adapted fluorescence parameters, measured as a function of time of exposure at four irradiances (220, 420, 1350, and 2300 μmol photons m^{-2} s^{-1}), were comparable with those in Figure 1 (data not shown). After 10 h of treatment, there was less than 20% decline in dark-adapted F_v/F_m and less than 20% increase in F_o in the two lower irradiance treatments. Treatment at 1350 μmol photons m^{-2} s^{-1} led to a steady value of dark-adapted F_v/F_m at 0.4 to 0.5 within 100 min and remained there throughout the 10-h treatment; F_o doubled but more slowly than at the highest irradiance. At 2300 μmol photons m^{-2} s^{-1}, F_v/F_m declined to between 0.2 and 0.4 within 100 min and remained there; F_o doubled but more rapidly than at 1350 μmol photons m^{-2} s^{-1}. As had been observed in the short-term experiments, there was no effect of treatment irradiance on the labeling of D1 protein by L-[^{35}S]Met (Fig. 4).

Photosynthesis and fluorescence quenching were monitored during the first 6 h of these treatments, yielding data generally consistent with those shown in Figure 2, i.e. at growth irradiance there were no changes in photosynthetic properties or fluorescence-quenching coefficients when initial controls were compared to 1- and 6-h treatments (data not shown). In leaves transferred to 420 μmol photons m^{-2} s^{-1} there were only small changes in light-response curves of photosynthetic O_2 evolution, or q_P, after 6 h (Fig. 6, A and D), but q_N in weak light increased markedly following 6 h of exposure at this irradiance (Fig. 6C), and the efficiency of PSII in weak light was reduced a little (Fig. 6B).

Treatment at 1350 μmol photons m^{-2} s^{-1} for 1 h led to a reduction in the apparent quantum yield but not the maximum rate of O_2 evolution (Fig. 6E). The maximum rate was decreased after 6 h, indicative of early photoacclimation to the higher irradiance, in spite of persistent lowering of the quantum yield of O_2 evolution and efficiency of PSII (Fig. 6F). The light response of q_P was not altered by these treatments (Fig. 6H), but within 1 h there was a marked and sustained increase in q_N measured at low irradiance (Fig. 6G) and a 30 to 40% decline in efficiency of PSII measured at low irradiance (Fig. 6F) consistent with results shown in Figure 2. These tendencies were even more evident in 2200 μmol photons m^{-2} s^{-1} treatments. After only 20 min there was a pronounced decline in the apparent quantum yield of photosynthetic O_2 evolution without a change in maximum rate, but within 3 h both parameters were massively depressed (Fig. 6I). Again, the light response of q_P was not much affected by this treatment, but q_N increased dramatically (Fig. 6K), especially at low light, consistent with a large decline in PSII efficiency (Fig. 6J). The decline in efficiency of PSII as indicated by the decline in dark-adapted F_v/F_m was well correlated with the percentage of functional PSII centers measured by O_2 flash yield (Fig. 7).

Changes in xanthophyll pigments resembled those observed previously (Fig. 3). There was no interconversion to zeaxanthin at the growth irradiance, and within 1 h only 18% of the violaxanthin was converted to antheraxanthin and zeaxanthin at 420 μmol photons m^{-2} s^{-1}, compared with 53% at 1350 and 2300 μmol photons m^{-2} s^{-1} (Table I).

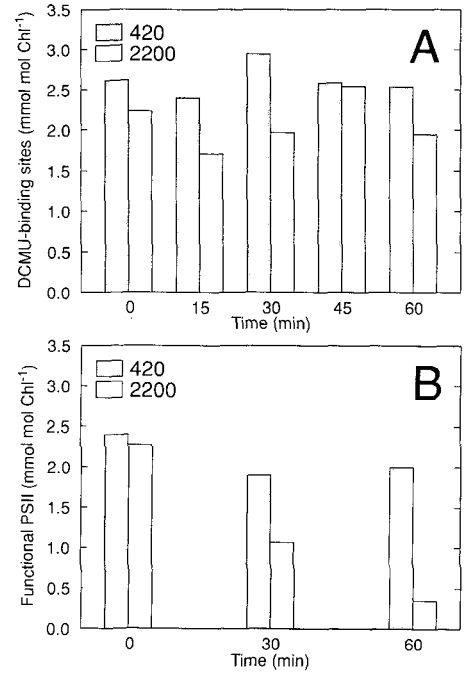

Figure 5. Changes in the concentrations of DCMU-binding sites (A) and functional PSII centers (B) in *Arabidopsis* leaves exposed to saturating or excess irradiance (420 or 2200 μmol photons m^{-2} s^{-1}, respectively). Note that initially the concentration of PSII centers determined by these two methods is comparable.

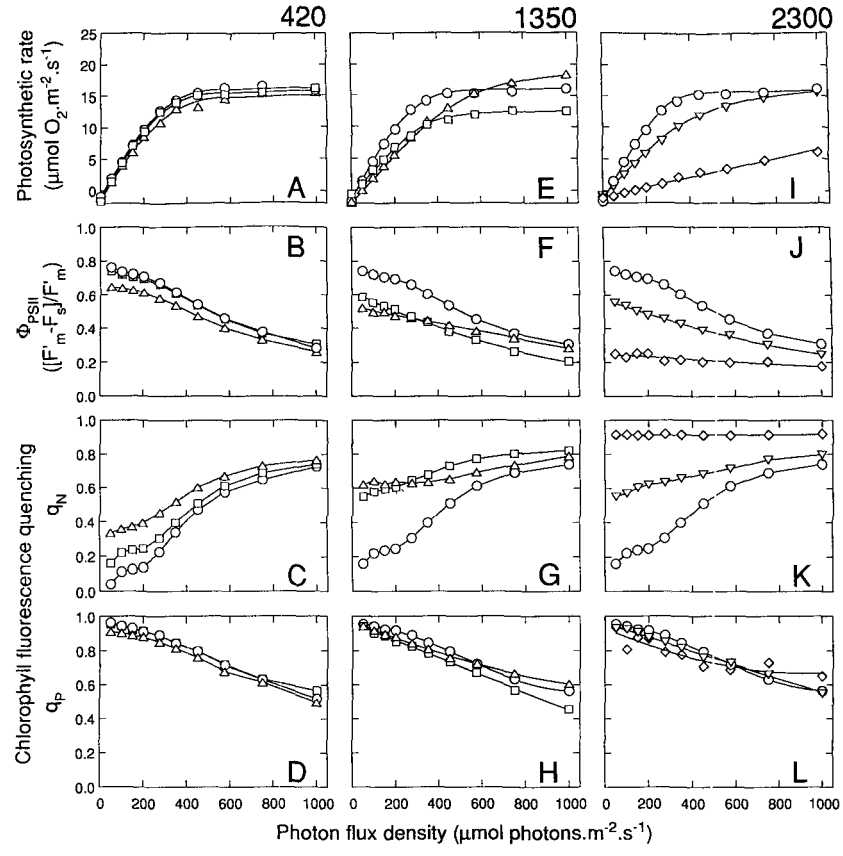

Figure 6. Light-response curves for CO_2-saturated photosynthesis (A, E, and I), PSII efficiency (B, F, and J), q_N (C, G, and K), and q_P (D, H, and L) in *Arabidopsis* leaves pretreated for 2 h at 220 μmol photons m^{-2} s^{-1} before exposure to saturating (A–D) or excess PPFD at 6× (E–H) or 10× (I–L) growth irradiance (420, 1350, or 2300 μmol photons m^{-2} s^{-1}, respectively). Data were obtained at zero time before the changeover in each case (○), then after 1 h (□) and 6 h (△) in series A to D and E to H, and after 20 min (▽) and 3 h (◇) in series I to L.

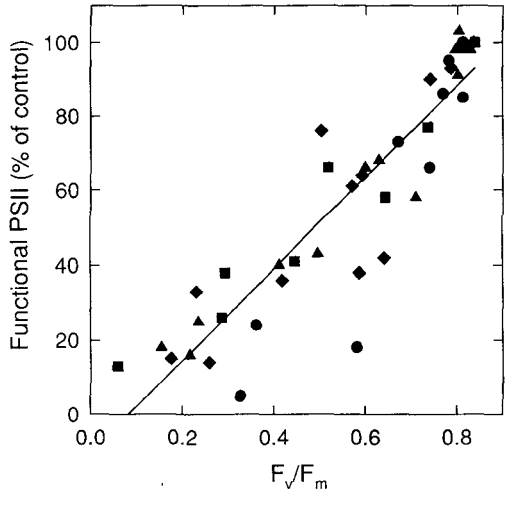

Figure 7. Relationships between changes in functional PSII center concentration and decline in dark-adapted F_v/F_m in *Arabidopsis*. Measurements were made on the same leaf discs in each case. Data are drawn from experiments (indicated by different symbols) described in Figures 1, 2, and 7 as well as additional repeat experiments undertaken by undergraduate students listed in the acknowledgments (r^2 = 0.821; y = 122.6x − 10.0).

After 10 h of exposure the xanthophyll cycle pools nearly doubled in each of the highest irradiance treatments, with all of the additional xanthophylls present in the de-epoxidated form (Table I).

The longer times used in these experiments and the comprehensive quantitative estimation of D1 protein using two different methods extended the observations shown in Figure 5. Figure 8, A and B, shows that at growth irradiance and 420 μmol photons m^{-2} s^{-1} there were no major negative changes in the normalized concentrations of anti-

Table I. *Effects of irradiance and exposure time on xanthophyll cycle pigment composition in Arabidopsis leaves from plants grown at 220 μmol photons m^{-2} s^{-1} (means of triplicate analyses)*

Fraction	Irradiance	Time of Exposure (h)		
		0	1	10
	μmol photons m^{-2} s^{-1}			
Total xanthophyll cycle pigments[a] (mmol mol^{-1} Chl)	250	42.9	37.8	48.3
	420	42.9	41.9	49.1
	1350	42.9	35.9	61.3
	2200	42.9	35.7	63.8
Antheraxanthin plus zeaxanthin (mmol mol^{-1} Chl)	250	4.3	3.0	2.9
	420	4.3	6.1	15.6
	1350	4.3	19.3	49.2
	2200	4.3	18.5	51.3

[a] Violaxanthin plus antheraxanthin plus zeaxanthin.

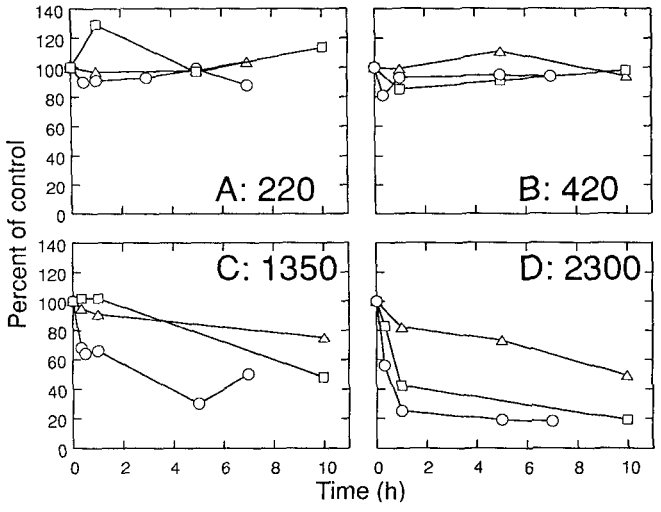

Figure 8. Time courses of changes in normalized concentrations of functional PSII centers (○), antibody-detectable D1 protein (□), and DCMU-binding sites (△) during exposure to growth irradiance (A), light saturation (B), 6× growth irradiance (C), or (D) 10× growth irradiance (220, 420, 1350, or 2300 μmol photons m^{-2} s^{-1}, respectively).

body-detectable D1 protein, [^{14}C]DCMU-binding sites, or functional PSII centers measured by O_2 flash yield. At 1350 μmol photons m^{-2} s^{-1} the concentration of [^{14}C]DCMU-binding sites declined very slowly over 10 h, whereas that of D1 protein remained unchanged for 1 h and subsequently declined to 50%. In contrast functional PSII centers declined by 30% in the 1st h and remained at about 20 to 40% in the 5- to 10-h treatments (Fig. 8C). At the highest irradiance all three measures of PSII reaction center properties declined, but the sequence was similar to that shown in Figure 8C, and the initial phases of the time courses were more rapid. Thus, [^{14}C]DCMU-binding site concentration declined more slowly than D1 protein concentration and this in turn was slower than the decline in functional PSII center concentration (Fig. 8D). After 5 h at 2300 μmol photons m^{-2} s^{-1} these parameters were only marginally more depressed than those after 10 h at 1350 μmol photons m^{-2} s^{-1}. After 10 h at the highest irradiance the concentration of antibody-detectable D1 protein equaled that of functional PSII centers at 7 h, but [^{14}C]DCMU-binding site concentrations remained more than twice as great. Again, there was no change in the $K_{1/2}$ for DCMU binding.

DISCUSSION

These comprehensive experiments permit, perhaps for the first time, an insight into the catena of interactive processes in photosynthesis, photoacclimation, photoprotection, and photoinhibition that have been researched in our laboratories, and those of many others, during the last decade. For the purposes of this discussion, and without becoming preoccupied for the moment with mechanisms, we accept that (a) the q_N indicates photoprotective processes, leading to dissipation of excitation energy as heat, of which at least one element is associated with the accumulation of antheraxanthin and zeaxanthin in thylakoid membranes (Demmig et al., 1987; Bilger and Björkman, 1990; Horton et al., 1991, Krause and Weis, 1991; Demmig-Adams and Adams, 1992); (b) the q_P indicates the extent of Q_A reduction associated with functional PSII complexes and that highly reduced Q_A pools have been suggested to potentiate processes of photoinhibitory damage (Krause, 1988; Krause and Weis, 1991; Prásil et al., 1992; Aro et al., 1993b); (c) both of the above processes lower the efficiency of PSII and the balance, capacity, and duration of these processes determine the extent, reversibility, and functional consequences of the down-regulation of PSII (Öquist et al., 1992; Osmond et al., 1993; Critchley and Russell, 1994); (d) dark-adapted Chl fluorescence parameters F_v/F_m and changes in F_o may serve to indicate the extent, reversibility, and functional consequences of changes in PSII efficiency (Krause, 1988; Franklin et al., 1992; Öquist et al., 1992; Osmond, 1994); (e) slowly reversible, extensive reduction in PSII efficiency (signaled by low values of dark-adapted F_v/F_m and elevated F_o) reflects some functional dynamic of the D1 protein of the reaction center (Osmond, 1994).

The *Arabidopsis* plants used here were grown under about 10% full sunlight, somewhat higher light intensity than commonly used for laboratory culture of these plants but nevertheless close to natural shade in average summer sunlight. Our experiments show that all photosynthetic parameters measured remain stable throughout 6 to 10 h at this growth irradiance. These processes also remain relatively stable when irradiance is doubled and photosynthesis is saturated. Thus, the light dependence of photosynthetic O_2 evolution and of q_P remains unchanged throughout. Nevertheless, the incomplete interconversion of xanthophylls at light saturation and the small (10–20%) increase in the zeaxanthin content of these plants after 10 h are correlated with more rapid engagement of q_N at low irradiance and with a small reduction in PSII efficiency indicated by fluorescence analysis in actinic light. Dark-adapted fluorescence shows that these changes relax quickly. Little consistently detectable change in the concentrations of functional PSII centers, D1 pool size, or DCMU-binding sites was observed, even after prolonged (6–10 h) illumination. Under these conditions the reduction state of the Q_A pool did not decrease below about 20% (q_P = 0.8) and q_N averaged 25 to 50% of its maximum value. The ratios of q_P/q_N during these treatments were always greater than 1.0, indicating a balance between a low Q_A reduction state and weak engagement of photoprotective processes that together support high photosynthetic efficiency. It is remarkable that this relative stability of all functional and structural parameters of leaf photosynthetic and PSII performance persists against a background of maximum rates of D1 protein turnover as measured by rates of incorporation of radiolabeled amino acid.

At higher irradiances excess photon absorption had marked effects on the composition and function of PSII.

These changes, leading to sustained reduction in the efficiency of PSII (Figs. 2F and 6J) occurred when the ratio of q_P/q_N was less than 1.0. For example, at 1350 μmol photons m^{-2} s^{-1} (Fig. 6, G and H), this ratio was about 1.0 at the start of the experiment and somewhat less after 6 h, because of an increase in q_N. The increase in q_N at low irradiance following exposure to excess photons appears to reflect the transformation of more than half of the violaxanthin pool to antheraxanthin and zeaxanthin (Fig. 3B; Table I). This is consistent with the notion that both zeaxanthin and ΔpH are essential for engagement of q_N (Gilmore and Yamamoto, 1991; Ruban et al., 1993). The sustained high q_N observed in our experiments could also be due to sustained adenylate charge (Gilmore and Björkman, 1994) or a photoinhibitory component of q_N. The light-response curves do not extend far enough to define q_P at 2200 μmol photons m^{-2} s^{-1}, either in controls or after treatments. In spite of the increased, sustained engagement of q_N, irradiance-dependent changes in q_P are very similar for all light treatments.

The loss of PSII efficiency is well portrayed by the decline in F_v/F_m and increase in F_o of dark-adapted fluorescence and correlates well with the loss of PSII reaction center function measured by O_2 flash yield, as pointed out previously (Chow et al., 1989; Öquist et al., 1992). At 1350 μmol photons m^{-2} s^{-1}, there was no net loss of D1 protein until between 5 and 10 h of treatment, but substantial reduction of PSII center efficiency and functional PSII occurred. At 2200 μmol photons m^{-2} s^{-1}, the rate of decline in PSII center efficiency also exceeded the net loss of D1 protein. Significantly, in both treatments the decline in DCMU-binding site concentration was much slower and D1 protein turnover was not accelerated. These measures of the turnover rates and concentrations of D1 protein and the measures of PSII center function suggest the following catena of processes during photoinhibition in vivo: A significant decrease in PSII reaction center efficiency occurs rapidly in *Arabidopsis* when excess photons are absorbed at 6 and 10× growth irradiance. We cannot easily distinguish whether the decline in the percentage of functional PSII centers is due to partial down-regulation of all, or complete down-regulation of some, PSII centers. However, the nature of the change in the light-response curves of photosynthetic O_2 evolution in leaves (Figs. 2E and 6G) and of O_2 flash yield in down-regulated PSII centers (Öquist et al., 1992) favors the complete down-regulation of a proportion of the PSII center population (Osmond, 1994).

Changes in the D1 protein in down-regulated PSII may follow, and apparent net loss of D1 protein is observed at prolonged excess irradiances. Because there was no difference in the synthesis of D1 protein in any of these treatments, consistent with experiments on several other species under similar conditions (Critchley et al., 1992; Geiken et al., 1992; Syme et al., 1992), the role of D1 protein turnover in leaves remains obscure.

The discrepancy between the changes in D1 protein concentration measured by antibody binding and DCMU binding reveals the complexity of D1 degradation (turnover) in vivo. It has been shown that D1 protein degradation in vitro may occur by cleavage on either the luminal or the stromal side of the thylakoid membrane (Barber and Andersson, 1992; Aro et al., 1993b). Cleavage on the stromal side, between transmembrane helices IV and V, should result in loss of DCMU-binding sites (Greenberg et al., 1987; De La Rivas et al., 1993) and, if this was the only site of proteolysis, one would expect equivalence in the antibody-detectable D1 protein and DCMU-binding site concentrations. Some studies of low-temperature photoinhibition of peas in vitro seem to indicate this form of acceptor-side proteolysis (Shipton and Barber, 1994), but other studies of spinach under these conditions (Chow et al., 1989) show that DCMU-binding site concentrations can remain well above functional PSII center concentrations. However, if cleavage occurs on the luminal side, between transmembrane helices I and II (Shipton and Barber, 1991), the resulting 23-kD fragment might continue to bind DCMU. It is not readily apparent why this fragment does not show up on autoradiographs and western blots. Nevertheless, our data may suggest that, in the course of photoinhibition in *Arabidopsis* in vivo under 10× growth irradiance, proteolysis of D1 protein, following loss of PSII center function, preferentially occurs on the luminal, or donor, side of the PSII reaction center, leaving many DCMU-binding sites intact. However, it is evident from the partial loss of DCMU-binding site concentration that stromal side proteolysis also occurs. Concurrent donor- and acceptor-side proteolysis can be induced in isolated PSII centers (Friso et al., 1993).

Our data confirm earlier suggestions that down-regulation of PSII to a nonfunctional, potentially dissipative center precedes turnover of the D1 protein (Krieger and Weis, 1992; Öquist et al., 1992; van Wijk and van Hasselt, 1993). These down-regulated PSII centers also seem to serve as dissipative centers when active centers are closed, and their slightly lower trapping efficiency is reflected in the increase in dark-adapted F_o, which commonly precedes and is then sustained during photoinhibition (Giersch and Krause, 1991; Anderson and Aro, 1994; Critchley and Russell, 1994). Further research is needed to clarify the mechanisms involved, but our observations help identify the processes of down-regulation of PSII centers and D1 protein turnover with a catena of events ranging from dynamic photoprotection to photoinhibition during exposure to photon excess.

ACKNOWLEDGMENTS

We wish to thank John Mullett for his gift of D1 antibodies, as well as Liza Apps, Vanessa Gillespie, Vikki Fisher, Danny Hawke, and Maria Marco for technical assistance in different experiments.

Received August 9, 1994; accepted November 15, 1994.
Copyright Clearance Center: 0032–0889/95/107/0943/10.

LITERATURE CITED

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

Aro E-M, McCaffery S, Anderson JM (1993a) Photoinhibition and D1 protein degradation in peas acclimated to different growth irradiances. Plant Physiol **103:** 835–843

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

Baker NR, Horton P (1987) Chlorophyll fluorescence quenching during photoinhibition. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Photoinhibition. Elsevier, Amsterdam, The Netherlands, pp 145–168

Barber J, Andersson B (1992) Too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci **17:** 61–66

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedra canariensis*. Photosynth Res **25:** 173–197

Bilger W, Schreiber U (1986) Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. Photosynth Res **10:** 303–310

Björkman O (1987) Low temperature chlorophyll fluorescence in leaves and its relationship to photon yield of photosynthesis in photoinhibition *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Photoinhibition. Elsevier, Amsterdam, The Netherlands, pp 123–144

Bradbury M, Baker NR (1986) The kinetics of photoinhibition of the photosynthetic apparatus in pea chloroplasts. Plant Cell Environ **9:** 289–297

Chow WS, Hope AB, Anderson JM (1990) A reassessment of the use of herbicide binding to measure photosystem II reaction centres in plant thylakoids. Photosynth Res **24:** 109–113

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

Chow WS, Osmond CB, Huang L-K (1989) Photosystem II function and herbicide binding sites during photoinhibition of spinach chloroplasts *in-vivo* and *in-vitro*. Photosynth Res **21:** 17–26

Critchley C (1988) The chloroplast thylakoid membrane system is a molecular conveyor belt. Photosynth Res **19:** 265–276

Critchley C, Russell AW (1994) Photoinhibition of photosynthesis *in vivo*: the role of protein turnover in photosystem II. Physiol Plant **92:** 188–196

Critchley C, Russell AW, Bolhàr-Nordenkampf HR (1992) The relationship between D1 turnover and photosynthesis. Photosynthetica **27:** 183–190

De La Rivas, Shipton CA, Panticos M, Barber J (1993) Acceptor side mechanism of photoinduced proteolysis of the D1 protein in photosystem II reaction centers. Biochemistry **32:** 6944–6950

Demmig-Adams B, Adams WW III (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol **43:** 599–626

Demmig B, Winter K, Krüger A, Czygan F-C (1987) Photoinhibition and zeaxanthin formation in intact leaves. A possible role for the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol **84:** 218–224

Franklin LA, Levavasseur G, Osmond CB, Henley WJ, Ramus J (1992) Two components of onset and recovery during photoinhibition of *Ulva rotundata*. Planta **187:** 399–408

Friso G, Giacometti GM, Barber J, Barbato R (1993) Evidence for concurrent donor and acceptor side photoinduced degradation of the D1 protein in isolated reaction centres of photosystem II. Biochim Biophys Acta **1144:** 265–270

Geiken B, Critchley C, Bolhàr-Nordenkampf HR, Renger R (1992) Synthesis of the photosystem II reaction centre protein D2 and the core antenna proteins CP43 and CP 47 as a function of light. Photosynthetica **27:** 201–206

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta **990:** 87–92

Giersch C, Krause GH (1991) A simple model relating photoinhibitory fluorescence quenching in chloroplasts to a population of altered photosystem II reaction centers. Photosynth Res **30:** 115–121

Gilmore AM, Björkman O (1994) Adenine nucleotides and the xanthophyll cycle in leaves. II. Comparison of the effects of CO_2- and temperature-limited photosynthesis on photosystem II fluorescence quenching, adenylate energy change and violaxanthin de-epoxidation in cotton. Planta **192:** 537–544

Gilmore AM, Yamamoto HY (1991) Resolution of lutein and zeaxanthin using a non-endcapped lightly-loaded C_{18} high-performance liquid chromatographic column. J Chromatogr **543:** 137–145

Greenberg BM, Gaba V, Mattoo AK, Edelman M (1987) Identification of a primary *in-vivo* degradation product of the rapidly turning-over, 32kd protein of photosystem II. EMBO J **6:** 3865–3869

Horton P, Ruban AV, Rees D, Pascal AA, Noctor GD, Young AJ (1991) Control of the light harvesting function of the LHCII chlorophyll protein complex. FEBS Lett **290:** 298–302

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

Krause H, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol **42:** 313–349

Krieger A, Weis E (1992) Energy-dependent quenching of chlorophyll-a fluorescence: the involvement of proton-calcium exchange at photosystem II. Photosynthetica **27:** 89–98

Kyle DJ, Ohad I, Arntzen CJ (1984) Membrane protein damage and repair: selective loss of a quinone-protein function in chloroplast membranes. Proc Natl Acad Sci USA **81:** 4070–4074

Long SP, Humphries S, Falkowski P (1994) Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Plant Mol Biol **45:** 633–662

Öquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta **187:** 450–460

Osmond CB (1994) What is photoinhibition: some insights from comparisons of sun and shade plants. *In* NR Baker, JR Bowyer, eds, Photoinhibition: Molecular Mechanisms to the Field. Bios Scientific Publishers, Oxford, UK, pp 1–24

Osmond CB, Ramus J, Levavasseur G, Franklin LA, Henley WJ (1993) Fluorescence quenching during photosynthesis and photoinhibition of *Ulva rotundata* Blid. Planta **190:** 91–106

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards 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* J Barber, ed, The Photosystems: Structure, Function and μmolecular Biology, Elsevier, Amsterdam, The Netherlands, pp 295–348

Robinson SA, Lovelock CE, Osmond CB (1993) Wax as a mechanism for protection against photoinhibition—a study of *Cotyledon orbiculata*. Bot Acta **106:** 307–312

Ruban AV, Young AJ, Horton P (1993) Induction of non-photochemical energy dissipation and absorbance changes in leaves. Evidence for changes in the state of the light-harvesting system of photosystem II *in vivo*. Plant Physiol **102:** 741–750

Seaton GGR, Walker DA (1992) Validating chlorophyll fluorescence measures of efficiency: observations on fluorimetric estimation of photosynthetic rate. Proc R Soc Lond B Biol Sci **249:** 41–47

Shipton CA, Barber J (1991) Photoinduced degradation of the D1 polypeptide in isolated reaction centers of photosystem II: evidence for an autoproteolytic process triggered by the oxidizing side of the photosystem. Proc Natl Acad Sci USA **88:** 6691–6695

Shipton CA, Barber J (1994) *In-vivo* and *in-vitro* photoinhibition reactions generate similar degradation fragments of D1 and D2 photosystem II reaction centre proteins. Eur J Biochem **220:** 801–808

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

Sundby C, McCaffery S, Chow WS, Anderson JM (1992) Photosystem II function, photoinhibition and turnover of D1 protein at different irradiances in normal and atrazine-resistant plants with an altered Qb-binding site. Plant Physiol **103:** 105–113

Syme AJ, Bolhàr-Nordenkampf HR, Russell AW, Critchley C (1992) Light-induced D1 protein degradation and photosynthesis in sun and shade leaves. *In* N Murata, ed, Research in Photosynthesis, Vol 4. Kluwer Academic, Dordrecht, The Netherlands, pp 337–340

Tischer W, Strotmann H (1977) Relationship between inhibitor binding by chloroplasts and inhibition of photosynthetic electron transport. Biochim Biophys Acta **460:** 113–125

van Wijk KJ, van Hasselt PR (1993) Photoinhibition of photosystem II *in-vivo* is preceded by down-regulation through light-induced acidification of the lumen: consequences for the mechanism of photoinhibtion *in-vivo*. Planta **189:** 359–368

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

Weis E, Berry JA (1987) Quantum efficiency of photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta **894:** 198–208