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Evaluation of the role of State transitions in determining the efficiency of light utilisation for CO_2 assimilation in leaves

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Abstract

Wheat leaves were exposed to light treatments that excite preferentially Photosystem I (PS I) or Photosystem II (PS II) and induce State 1 or State 2, respectively. Simultaneous measurements of CO_2 assimilation, chlorophyll fluorescence and absorbance at 820 nm were used to estimate the quantum efficiencies of CO_2 assimilation and PS II and PS I photochemistry during State transitions. State transitions were found to be associated with changes in the efficiency with which an absorbed photon is transferred to an open PS II reaction centre, but did not correlate with changes in the quantum efficiencies of PS II photochemistry or CO_2 assimilation. Studies of the phosphorylation status of the light harvesting chlorophyll protein complex associated with PS II (LHC II) in wheat leaves and using *chlorina* mutants of barley which are deficient in this complex demonstrate that the changes in the effective antennae size of Photosystem II occurring during State transitions require LHC II and correlate with the phosphorylation status of LHC II. However, such correlations were not found in maize leaves. It is concluded that State transitions in C_3 leaves are associated with phosphorylation-induced modifications of the PS II antennae, but these changes do not serve to optimise the use of light absorbed by the leaf for CO_2 assimilation.

Abbreviations: Fm, Fo, Fv – maximal, minimal and variable fluorescence yields; Fm', Fv' – maximal and variable fluorescence yields in a light adapted state; LHC II – light harvesting chlorophyll *a/b* protein complex associated with PS II; q_P – photochemical quenching; ΔA_{820} – light-induced absorbance change at 820 nm; $\phi_{PS\,I}$, $\phi_{PS\,II}$ – relative quantum efficiencies of PS I and PS II photochemistry; ϕ_{CO_2} – quantum yield of CO_2 assimilation

Introduction

The absorption spectra of PS I and PS II are different with PS I and PS II being excited preferentially above and below 670 nm, respectively (Ried 1972). In the context of the Z-scheme for linear electron transport the mean rates of excitation of PS I and PS II reaction centre populations must be equal for a leaf to achieve the maximal quantum efficiency of water oxidation. While transient differences (in the order of seconds) in the excitation rates of the two photosystems may be tolerated, clearly any long term imbalance in the excitation of the two photosystem populations would cause a proportional decrease in the quantum efficiency. Such imbalances can be alleviated by modifying either the stoichiometry of PS I:PS II reaction centres or the effective antennae sizes of PS I and PS II or both. In the longer term (hours or days) light-induced changes in gene expression can result in changes in the relative amounts of PS I, PS II and the light-harvesting chlorophyll *a/b* protein complex associ-

ated with PS II (LHC II) which are considered to optimise light use efficiency in photosynthesis (Anderson 1986, Anderson and Osmond 1987, McKiernan and Baker 1991). In the shorter term (order of minutes) adjustments in the distribution of excitation energy between PS I and PS II populations is thought to be achieved by State transitions (Myers 1971, Fork and Satoh 1986, Williams and Allen 1987).

Murata (1969) and Bonaventura and Myers (1969) demonstrated independently that readjustments in the distribution of excitation energy between PS I and PS II could occur in vivo, and such changes became commonly known as State 1-State 2 transitions (Myers 1971). State 1 can be generated on equilibration of thylakoids in light absorbed preferentially by PS I and results from the photosynthetic apparatus adapting to direct a greater proportion of absorbed radiation to PS II than PS I. State 2 results on equilibration in light absorbed preferentially by PS II and a greater proportion of absorbed quanta are now directed to PS I than PS II. On transition from State 1 to State 2, it has been estimated for *Chlorella pyrenoidosa* that 10% of the absorbed quanta previously directed to PS II are redirected to PS I (Bonaventura and Myers 1969). The mechanistic basis of the State 1–State 2 transition is considered to be the phosphorylation of a proportion of the apoproteins of LHC II; this alters the net electrical charge on the complex and the interaction of LHC II complexes with each other and with PS II and PS I, and results in changes in the absorption cross sections of PS I and PS II (Allen 1992). These concepts have developed from many elegant experiments performed on isolated thylakoids and chloroplasts (for review see Allen 1992).

Although it has been demonstrated unequivocally that State transitions do occur in leaves (Chow et al. 1981, Canaani et al. 1982, 1984, Hodges and Barber 1983, Canaani and Malkin 1984, Webber et al. 1984, Malkin et al. 1986, Veeranjaneyulu et al. 1991), no convincing evidence exists to demonstrate that State transitions optimise the efficiency of light use for CO_2 assimilation by leaves. The aim of the present study is to examine changes in PS I and PS II photochemistry and in the quantum efficiency of CO_2 assimilation during State transitions in leaves. Recently non-invasive techniques have been developed to allow simultaneous measurements of CO_2 assimilation and the quantum efficiencies of photochemistry of the PS I and PS II populations in leaves (Harbinson and Woodward 1987, Harbinson and Hedley, 1988, Genty et al. 1989, 1990, Harbinson et al. 1989). In this study these techniques are used to monitor simultaneously these parameters in leaves undergoing State transitions. Data is presented for wheat and maize leaves and also for leaves of barley mutants deficient in LHC II. It is demonstrated that although State transitions are associated with changes in the effective antenna size of PS II, they do not appear to optimise the efficiency of light use for carbon assimilation.

Material and methods

Plant material and growth conditions

Winter wheat (*Triticum aestivum*) cv Avalon and maize (*Zea mays*) cv LG11 were grown in a glasshouse at 25/20 °C mean day/night temperature, supplemented with artificial light to give a minimum photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1} during a 16 h photoperiod. Barley (*Hordeum vulgare*) mutants deficient in LHC II were also grown under the same conditions. Wheat and barley were grown in F2 potting compost (Fisons Ltd., Ipswich, UK) and maize was grown in a 50:50 mix of F2 potting compost and perlite. All plants were watered daily with Hoagland's nutrient solution. The second fully expanded leaf of all plants was used in all experiments.

To study the degree of LHC II phosphorylation in vivo wheat and maize plants were grown in solution culture containing $^{32}PO_4^{3-}$. Seeds were germinated in vermiculite and then transferred to 2 cm diameter glass test tubes containing 10 (for wheat) or 25 (for maize) cm^3 Hoagland's nutrient medium and 100 μCi $^{32}PO_4^{3-}$ (Amersham International, Amersham, UK). When the plants reached the 3 leaf stage (10 days after sowing for maize, 18 days for wheat), leaves of three plants were exposed to the experimental light treatments described below and then thylakoids were isolated for analysis.

Measurements of photosynthetic efficiencies

Measurements of CO_2 assimilation, chlorophyll fluorescence and the light-induced absorbance change at 820 nm (ΔA_{820}) in leaves were made concurrently using equipment and techniques des-

cribed previously (Genty et al. 1989, 1990, Harbinson and Hedley 1989, Harbinson et al. 1989). Measurements on wheat and barley leaves were made under non-photorespiratory conditions, i.e. in a gas phase containing 380 ppm CO_2 and 2% O_2 in N_2. Measurements on maize leaves were made under standard atmospheric conditions (350–400 ppm CO_2 and 21% O_2 in N_2).

Chlorophyll fluorescence signals were analysed as described by Genty et al. (1989) to provide estimates of the relative quantum efficiency of PS II photochemistry ($\phi_{PS\,II}$), the efficiency of excitation energy capture by *open* PS II reaction centres (Fv'/Fm') and photochemical quenching (q_P); $\phi_{PS\,II}$ equates to the product of Fv'/Fm' and q_P. The recently suggested nomenclature for chlorophyll fluorescence parameters (van Kooten and Snel 1990) is adopted throughout.

The ΔA_{820} generated in a leaf on removal of the actinic light was used to provide an estimate of the relative quantum efficiency of PS I photochemistry ($\phi_{PS\,I}$). The rapid decay (over ca. 200 ms) of 820 nm absorbance (ΔA_{820}) on removal of the actinic light corresponds to the rapid reduction of the PS I reaction centre, P700, in the dark and can be used to estimate the redox state of the P700 pool that existed in the presence of actinic light (Harbinson and Woodward 1987, Harbinson and Hedley 1988). The relative amount of non-oxidised P700 in a leaf at any given time, which is directly proportional to $\phi_{PS\,I}$, was determined from $(1-\Delta A_{820}/\Delta A_{820max})$. The ΔA_{820} value corresponding to the maximal oxidation of P700 (ΔA_{820max}) of each leaf was determined routinely by exposing the leaf to far red radiation (PFD 12 μmol m^{-2} s^{-1}), which is preferentially absorbed by PS I relative to PS II. The values for ΔA_{820max} estimated from the exposure to this far red radiation were found to be similar (always $\ll$ 5% difference) to ΔA_{820max} determined from analysis of the ΔA_{820} values produced from actinic light dosage response curves. The values of ΔA_{820} were plotted against the reciprocal of a range of actinic of irradiances used to produce a photon flux density dosage response curve for the leaf, and the ΔA_{820max} determined by extrapolation of the linear portion of the plot to infinite photon flux density.

The quantum efficiency of CO_2 assimilation (ϕ_{CO_2}) of leaves was calculated by dividing the rate of CO_2 assimilation (corrected for respiratory losses by addition of the dark rate of respiration to the net assimilation rate) of the irradiated leaf area by the rate at which quanta were absorbed by this area. Measurements of leaf absorbance in any given light environment were made using a Taylor integrating sphere in a Cary 210 spectrophotometer (Rackham and Wilson 1968). All experiments were replicated with a minimum of 3 leaves.

In all experiments the leaf was dark adapted for at least 45 min prior to determination of the minimal level of fluorescence (Fo) by a weak modulated measuring beam (> 0.1 μmol m^{-2} s^{-1}). The maximal fluorescence level (Fm) was then determined by exposing the leaf to a 2 s saturating flash (< 10 000 μmol m^{-2} s^{-1}). State 2 was induced in the leaves by exposure to Light 2 for 45 min; Light 2 consisted of actinic white light (PPFD 35 μmol m^{-2} s^{-1}) produced from a quartz halogen source supplemented with red light of 650 nm (PFD 55 μmol m^{-2} s^{-1}) produced from a quartz halogen source in conjunction with a 650 nm Ealing interference filter (half band width 5.7 nm). To generate State 1 the 650 nm red light was replaced with 710 nm far-red radiation (PFD 12 μmol m^{-2} s^{-1}) produced from a quartz halogen source in conjunction with a 710 nm Ealing interference filter (half band width 6.2 nm); this white light enhanced with far red radiation is subsequently referred to as Light 1.

Thylakoid isolation and polypeptide analysis

Immediately after a given light treatment leaves were ground for 15 s in a pre-chilled mortar and pestle in 10 cm^3 of isolation buffer (400 mM sucrose, 50 mM tricine/KOH, 5 mM $MgCl_2$ and 10 mM NaF at pH 7.6). Fluoride was added to inhibit any phosphatase activity. The slurry was filtered through 2 layers of miracloth (Calbiochem) and centrifuged for 2 min at 3000g in a Sorvall SS-34 rotor. All subsequent steps were carried out at 4 °C. The pellet was resuspended in a small volume of isolation buffer, recentrifuged and resuspended in a small volume of a second buffer containing: 10 mM tricine/KOH (pH 7.0), 5 mM NaEDTA and 10 mM NaF. SDS-polyacrylamide gel electrophoresis of thylakoid polypeptides in 10–18% acrylamide gels using the buffer system of Laemmli (1970) and autoradiography were performed essentially as described previously (Nie and Baker 1991). Aliquots of solubilised thylakoids containing 20 μg chlorophyll were loaded onto each gel track.

Results

When examining the effects of State transitions on the efficiency of light use for photosynthesis care must be exercised to avoid complications arising due to concomitant changes in the efficiency of PS II photochemistry as a result of light intensity-dependent, non-photochemical quenching of PS II excitation energy, commonly referred to as *down-regulation*. Large changes in such excitation energy quenching occur over regions of the light dosage response curve for photosynthesis where increases in PPFD are not accompanied by a directly proportional increase in CO_2 assimilation, and contribute to the light intensity-dependent depressions in the quantum efficiency of CO_2 assimilation (Baker and Ort 1992). Consequently, experiments were conducted at PPFDs in the region of the light dosage response curve where CO_2 assimilation increases linearly with increasing PPFD and is not modified by changes in light intensity dependent changes in non-photochemical quenching of excitation energy.

In order to induce State 1 and State 2, leaves were exposed to two light regimes of white light (PPFD of 35 μmol m^{-2} s^{-1}) supplemented with either red (PFD 55 μmol m^{-2} s^{-1}) or far red (PFD 12 μmol $m^{-2}s^{-1}$) radiation. These light treatments are referred to as Light 2 and Light 1, respectively, and are defined in the 'Materials and methods' section. Light 2 will preferentially excite PS II and result in State 2 at steady state; Light 1 preferentially excites PS I and produces State 1 at steady state. The classical fluorescence transients indicative of State transitions (Bonaventura and Myers 1969, Allen 1992) are observed when leaves are exposed to these two light regimes (Fig. 1). Exposure of a dark-adapted wheat leaf to Light 2 induces a normal fluorescence induction curve. Switching from Light 2 to Light 1 when the leaf is at steady state photosynthesis results in an immediate quenching of fluorescence yield followed by a gradual increase to a constant yield that is indicative of a transition from State 2 to State 1. Switching from Light 1 back to Light 2 produces an immediate increase in fluorescence yield followed by a gradual decline to a steady state yield as a transition occurs from State 1 back to State 2.

The changes in quantum efficiency of CO_2 assimilation (ϕ_{CO_2}) and the quantum efficiencies of photochemistry of the PS I ($\phi_{PS\,I}$) and PS II ($\phi_{PS\,II}$) populations of wheat leaves that occur on switching between Light 2 and Light 1 are shown in Fig. 2. As expected in Light 2 the leaf had a high $\phi_{PS\,I}$, due to the preferential excitation of PS II, that dropped immediately on switching to Light 1 as the rate of excitation of PS I is increased relative to that of PS II. The immediate increase in $\phi_{PS\,II}$ on switching from Light 2 to Light 1 is due to a rapid oxidation of the PS II electron acceptors, indicated by a large increase in q_P. Genty et al. (1989) have shown that $\phi_{PS\,II}$ is determined by the product of the efficiency of excitation energy capture by *open* PS II reaction centres (Fv'/Fm') and q_P. These changes are also accompanied by a decrease in ϕ_{CO_2} as PS I is preferentially excited on switching to Light 1, presumably due to the large decrease in the efficiency of PS I photochemistry that results from an increased proportion of PS I excitation energy being dissipated by non-radiative decay. After these predicted rapid changes and during the transition from State 2 to State 1, both $\phi_{PS\,I}$ and $\phi_{PS\,II}$ slowly increase, whereas ϕ_{CO_2} slowly decreases then increases slightly. The increase in $\phi_{PS\,II}$ on transition from State 2 to State 1 is due to an increase in Fv'/Fm', since q_P does not change significantly, and is consistent with an increase in the effective antennae size of PS II. However, the increase in $\phi_{PS\,I}$ is presumably due to an increased supply of electrons to PS I from PS II since the absorption cross section of PS I would be expected to decrease rather than increase under these conditions. On switching back from Light 1 to Light 2, both ϕ_{CO_2} and $\phi_{PS\,I}$ return immediately to the high values previously achieved in Light 2; however, $\phi_{PS\,II}$ drops to a value well below that found previously at steady state in Light 2. Since a large drop in q_P accompanies this decrease in $\phi_{PS\,II}$ with only a small decrease in Fv'/Fm', a rapid reduction of PS II acceptors accounts primarily for these

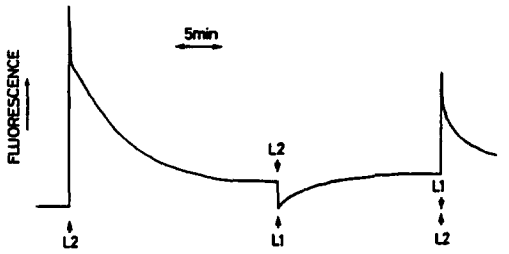

Fig. 1. Changes in fluorescence yield when a wheat leaf is exposed to Light 2 (L2) and Light 1 treatments (L1). ↑ indicates light switched on, ↓ indicates light switched off.

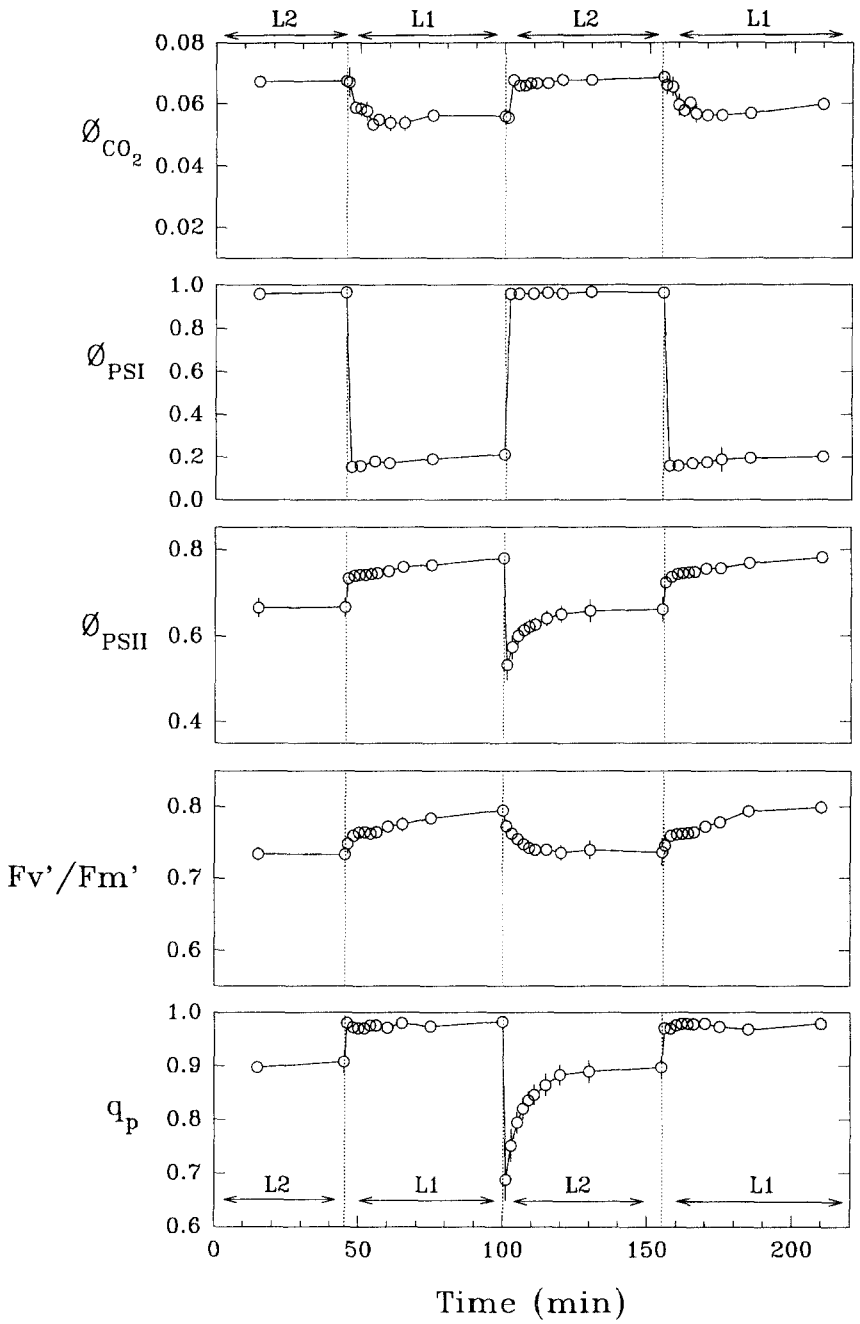

Fig. 2. Changes in the quantum efficiencies of CO_2 assimilation (ϕ_{CO_2}) and PS I ($\phi_{PS\,I}$) and PS II photochemistry ($\phi_{PS\,II}$), the efficiency of excitation energy capture by *open* PS II reaction centres (Fv'/Fm') and photochemical quenching (q_P) by PS II during sequential exposures of wheat leaves to Light 2 (L2) and Light 1 (L1) treatments. Data are the means of 3 replicates and standard errors are shown when larger than the symbol.

changes. During the transition from State 1 to State 2, Fv'/Fm' decreases, as would be predicted as the effective antennae size of PS II decreases again. However, this decrease in Fv'/Fm' does not contribute to a decrease in $\phi_{PS\,II}$; $\phi_{PS\,II}$ increases during this period due to a large increase in q_P. Clearly, factors other than the efficiency of excitation energy transfer to the PS II reaction centres are determining the

flux of electrons through PS II. After reaching a steady state of photosynthesis in Light 2 switching again to Light 1 produced responses in ϕ_{CO_2}, $\phi_{PS\ I}$, $\phi_{PS\ II}$, Fv'/Fm' and q_P similar to those observed during the previous transition from State 2 to State 1.

The changes in ϕ_{CO_2}, $\phi_{PS\ II}$, Fv'/Fm' and q_P that are observed on switching from Light 2 to Light 1 and vice versa (Fig. 2) cannot be attributed to the differences in the total PFD absorbed by the leaves in the two light treatments. In a control experiment in which leaves on reaching steady state photosynthesis in 96 μmol m^{-2} s^{-1} of white light, which is equivalent to the PPFD absorbed by a leaf when exposed to Light 2, were then switched to a white light PPFD of 43 μmol m^{-2} s^{-1}, which is equivalent to the PFD absorbed by a leaf exposed to Light 1, no significant changes were observed in ϕ_{CO_2}, $\phi_{PS\ II}$, Fv'/Fm' or q_P (data not shown). Consequently, the changes in these parameters on switching between Lights 1 and 2 can be attributed to differential excitation of PS I and PS II due to the changing light quality and not to intensity-dependent changes.

The phosphorylation status of LHC II in the wheat leaves during the experiment shown in Fig. 2 was determined by growing plants in solution culture supplemented with $^{32}PO_4^{3-}$. On exposure to the same light treatments as shown in Fig. 2, leaves exhibited the same changes in the photosynthetic parameters. When the leaves had reached steady state photosynthesis in Light 2, Light 1 and then Light 2 again, thylakoids were isolated and the polypeptides profiles produced by SDS-PAGE. The autoradiograms of the polypeptide profiles (Fig. 3) demonstrate that LHC II is strongly phosphorylated at steady state in Light 2 (State 2), but only weakly phosphorylated at steady state in Light 1 (State 1). These data confirm that LHC II phosphorylation in the wheat leaves occurs in response to light preferentially exciting PS II and dephosphorylation accompanies the preferential excitation of PS I, as has been shown previously for leaves (Bennett, 1983, 1984a,b).

To examine the possible effects on State transitions of light-induced quenching *(down regulation)*, the changes in the photosynthetic parameters on supplementing Light 2 and Light 1 with increasing levels of white light were examined. The experiment shown in Fig. 2 was repeated using a range of PPFDs of white light (100–600 μmol m^{-2} s^{-1}) instead of using the single white light PPFD of 35 μmol m^{-2} s^{-1} as was the case for Light 1 and Light 2 treatments; the white light at each intensity was supplemented as for the experiment shown in Fig. 2 with either 55 μmol m^{-2} s^{-1} of red or 12 μmol m^{-2} s^{-1} of far-red light. Predictably for a leaf in State 2 the efficiencies of PS I and PS II photochemistry and CO_2 assimilation all decrease as the PPFD of white light is increased due to increased light intensity-dependent quenching of excitation energy (Fig. 4). At white light levels of 35, 100 and 200 μmol m^{-2} s^{-1} switching from the red to far-red treatment induced rapid drops of decreasing magnitude in ϕ_{CO_2} that were rapidly reversed on returning to the red light treatment. There are no indications at white light levels of 100 and 200 μmol m^{-2} s^{-1} that a mechanism is operating in the leaf to optimise CO_2 assimilation during the transition from State 2 to State 1. If changes were occurring to optimise CO_2 assimilation then a rise in ϕ_{CO_2} would be expected following the decrease in this parameter on switching from the red to far red treatment. The slow increases observed in Fv'/Fm' at all white light levels are consistent with the occurrence of a transition from State 2 to State 1 on switching from red to far-red treatment. Similarly, it would appear that the State 1 – State 2 transition on switching back from far-red to red treatment also occurs at all white light levels except for 600 μmol m^{-2} s^{-1}. It is of note that during the State 1– State 2 transition slow increases in $\phi_{PS\ II}$ occur at all white light levels, and these are clearly attributable to increases in q_P, since Fv'/Fm' decreases, and are not associated with changes in CO_2 assimilation. These increases in $\phi_{PS\ II}$ and q_P imply an increased electron flux through PS II due to a decrease in the resistance for electron transfer down stream of Q_A.

To further examine the role of LHC II in determining the changes in ϕ_{CO_2}, $\phi_{PS\ I}$, $\phi_{PS\ II}$, Fv'/Fm' and q_P in response to preferential excitation of PS I or PS II, mutants of barley lacking or deficient in LHC II were studied. The mutants were subjected to the light regimes shown in Fig. 2. Leaves of wild type barley showed responses in all of the photosynthetic parameters similar to those illustrated for wheat leaves in Fig. 2 (data not shown). The changes in the photosynthetic parameters occurring in the leaves of the *chlorina-f2* barley mutant that has no chlorophyll *b* were quite different to the wild type and are shown in Fig. 5. At steady state in Light 2 this mutant has a low maximal ϕ_{CO_2} of ca. 0.04, which

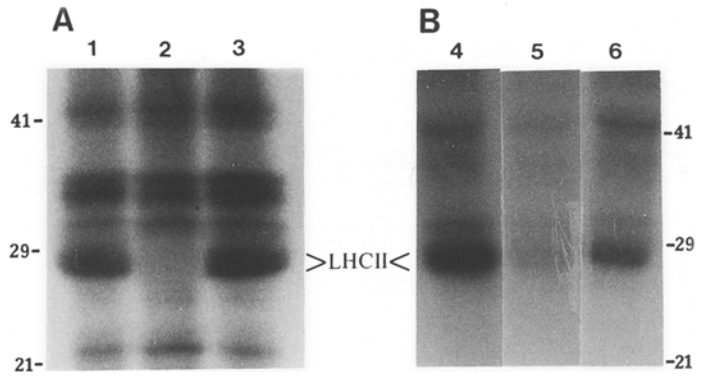

Fig. 3. Autoradiograms of the polypeptide profiles of thylakoids isolated from the leaves of wheat (A) and maize (B) plants that had been grown hydroponically in culture solution containing $^{32}PO_4^{3-}$ and exposed sequentially to Light 2 (tracks 1 and 4), Light 1 (tracks 2 and 5) and Light 2 again (tracks 3 and 6). Thylakoids were isolated after the leaves had reached steady state photosynthesis in each light treatment (ca. 50 min). Molecular masses of marker proteins are given in kDa to the side of the autoradiograms and the positions of LHC II polypeptides indicated.

drops immediately to ca. 0.03 on transfer to Light 1. Since the changes in $\phi_{PS\ II}$, Fv'/Fm' and q_P are negligible during the experiment, it is evident that the far-red induced decrease in ϕ_{CO_2} is due to the decrease in $\phi_{PS\ I}$ as PS I is preferentially excited. The increased rate of excitation of PS I on transfer from Light 2 to Light 1 results in increased electron flux through PS I, indicated by the decrease in $\phi_{PS\ I}$. However this increase in flux cannot contribute as efficiently to CO_2 assimilation as the electron flux through PS I in Light 2 does; if it did then ϕ_{CO_2} would remain constant. Presumably the increased electron flux through PS I, in the absence of any parallel decrease in $\phi_{PS\ II}$, must be attributable to cyclic electron flux around PS I. It should be noted that $\phi_{PS\ I}$ at steady state in Light 2 is considerably reduced (ca. 0.27) in the mutant compared to the wild type (0.96); this demonstrates that the predicted increased rate of excitation of PS I, relative to PS II, in the mutant compared to the wild type, is attributable to the reduction in the size of the PS II antennae. The absence of LHC II in the *chlorina-f2* mutant correlates with a loss of ability of the leaf to modify $\phi_{PS\ II}$, Fv'/Fm' and q_P in response to preferential excitation of PS I or PS II. Barley mutant *clo-f2*[109], which is only partially deficient in LHC II (chlorophyll *a/b* ratio of 6.8) relative to the wild type (Simpson et al. 1985), exhibits responses intermediate between the wild type and *chlorina-f2* mutant on switching between Light 2 and Light 1 (Fig. 5). Similar results were also obtained with two other barley mutants deficient in LHC II to varying extents (data not shown). Clearly LHC II does appear to play a role in determining the ability of thylakoids to undergo transitions in response to differential excitation of PS I and PS II that modify $\phi_{PS\ II}$, however such transitions are not associated with significant changes in ϕ_{CO_2}.

Finally, the response of maize leaves to switching between Light 2 and Light 1 was examined. Maize, being C_4, has different chloroplast populations in the mesophyll and bundle sheath tissues, which have different metabolic demands for the products of photosynthetic electron transport. Together the mesophyll and bundle sheath chloroplasts provide 5 ATP and 2 NADPH per CO_2 assimilated (Hatch 1987). However, in maize the majority of the PS II population is located in the mesophyll chloroplasts (Woo et al. 1970, Mayne et al. 1974), which contribute 3 ATP and 2 NADPH per CO_2 assimilated; the bundle sheath chloroplasts contribute the remaining 2 ATP per CO_2 assimilated by the leaf (Hatch 1987). Consequently, the response of the photosynthetic parameters of maize leaves to preferential excitation of PS I or PS II may be quite different to that found for C_3 leaves. The changes in ϕ_{CO_2}, $\phi_{PS\ I}$, $\phi_{PS\ II}$, Fv'/Fm' and q_P for maize leaves exposed to switches between Light 2 and Light 1 are shown in Fig. 6, and it is evident that these are quite different to those shown in Fig. 2 for wheat leaves. A major difference is that on the first switch from Light 2 to Light 1 only a very small decrease in ϕ_{CO_2} occurs and this is reversed within 10 min; in wheat (and wild type barley) ϕ_{CO_2} always decreases significantly. However, more surprisingly, on switching back to Light 2 from Light 1 there is a

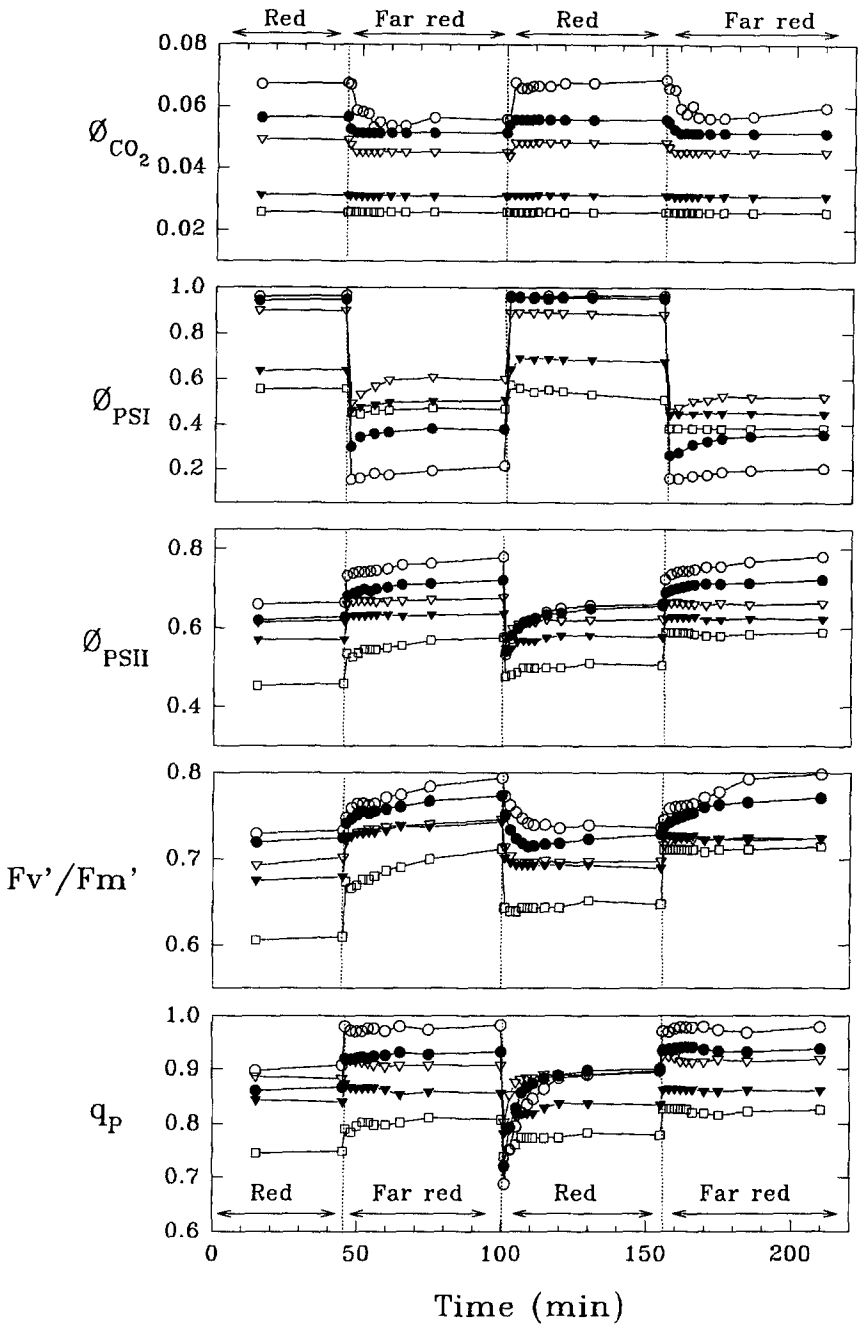

Fig. 4. Changes in the quantum efficiencies of CO_2 assimilation (ϕ_{CO_2}) and PS I ($\phi_{PS\,I}$) and PS II photochemistry ($\phi_{PS\,II}$), the efficiency of excitation energy capture by *open* PS II reaction centres (Fv'/Fm') and photochemical quenching (q_P) by PS II of wheat leaves exposed to a range of PPFDs of white light during sequential additions of red (650 nm, 55 μmol m^{-2} s^{-1}) and far red (710 nm, 12 μmol m^{-2} s^{-1}) light. PPFDs of white light used were 35 (○), 100 (●), 200 (▽), 400 (▼) and 600 (□) μmol m^{-2} s^{-1}. Data are the means of 3 replicates.

large drop in ϕ_{CO_2} in maize whereas in wheat ϕ_{CO_2} increased immediately back to the higher ϕ_{CO_2} found previously at steady state in Light 2. This decrease in ϕ_{CO_2} in maize is followed by a slow increase which is not associated with an increased electron flux through PS II since $\phi_{PS\,II}$ decreases. This decrease in $\phi_{PS\,II}$ is attributable to decreases in both q_P and Fv'/Fm'. On switching back again from Light 2 to

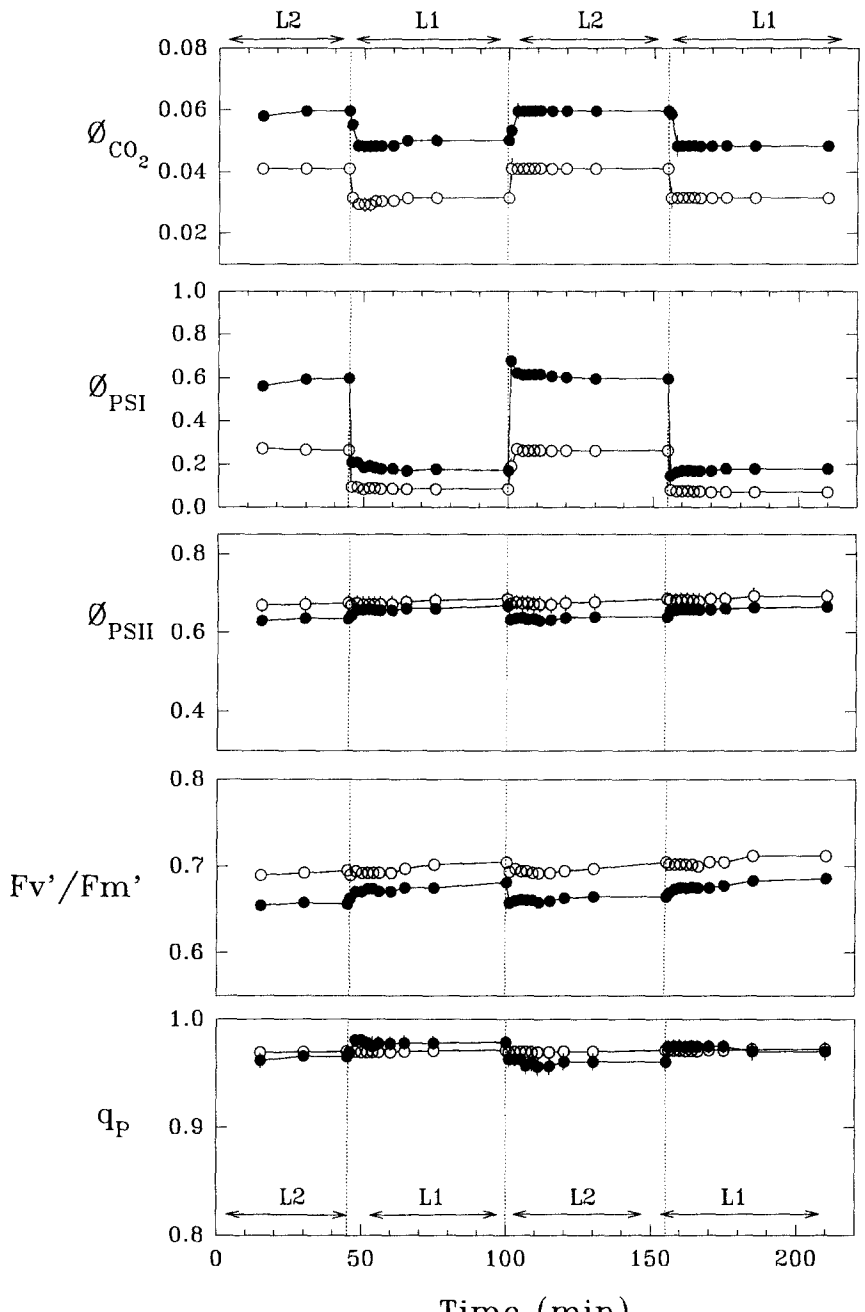

Fig. 5. Changes in the quantum efficiencies of CO_2 assimilation (ϕ_{CO_2}) and PS I ($\phi_{PS\,I}$) and PS II photochemistry ($\phi_{PS\,II}$), the efficiency of excitation energy capture by *open* PS II reaction centres (Fv'/Fm') and photochemical quenching (q_P) by PS II during sequential exposures of leaves of the barley mutants *chlorina-f2* (○) and *clo-f2*[109] (●) to Light 2 (L2) and Light 1 (L1) treatments. Data are the means of 3 replicates and standard errors are shown when larger than the symbol.

Light 1, ϕ_{CO_2} increases back to the level previously attained in Light 1. The phosphorylation status of LHC II in the maize leaves at steady state in these light regimes was monitored in plants grown in solution culture supplemented with $^{32}PO_4^{3-}$. The autoradiograms of thylakoid polypeptide profiles of thylakoids isolated from maize leaves at steady state in Light 2, Light 1 and on return to Light 2 are shown in Fig. 3. As with the wheat leaves, LHC II was heavily phosphorylated in Light 2 and after

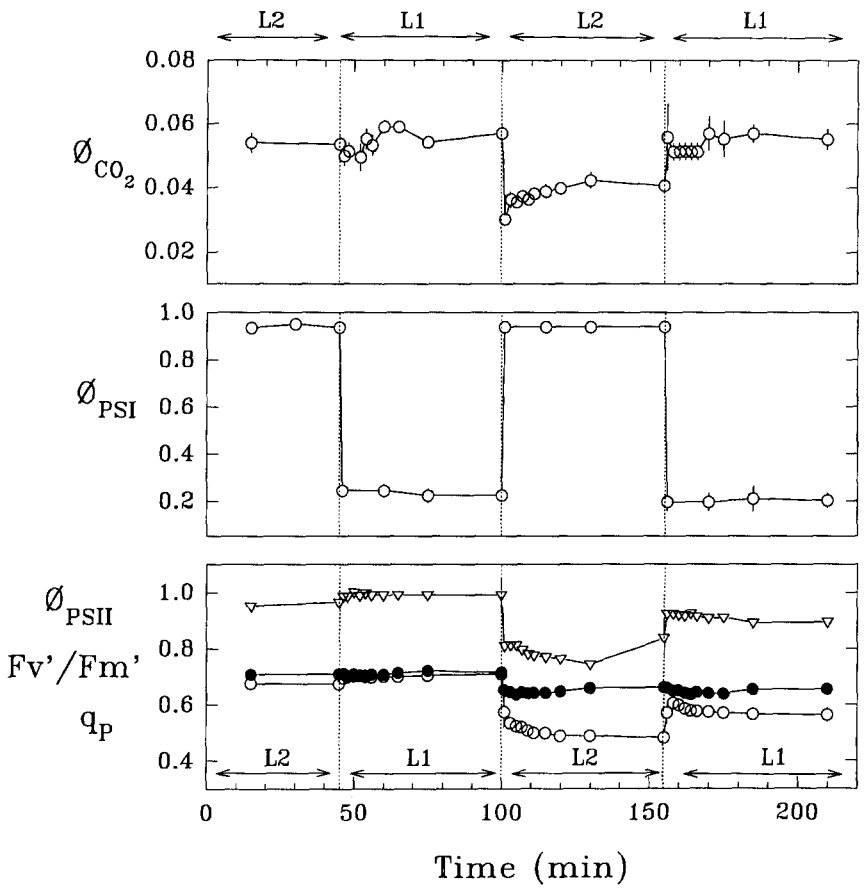

Fig. 6. Changes in the quantum efficiencies of CO_2 assimilation (ϕ_{CO_2}) and PS I ($\phi_{PS\,I}$) and PS II photochemistry ($\phi_{PS\,II}$, ○), the efficiency of excitation energy capture by *open* PS II reaction centres (Fv'/Fm', ●) and photochemical quenching (q_P, ▽) by PS II during sequential exposures of maize leaves to Light 2 (L2) and Light 1 (L1) treatments. Data are the means of 3 replicates and standard errors are shown when larger than the symbol.

transfer to Light 1 became dephosphorylated. On transfer from Light 1 back to Light 2 LHC II became phosphorylated again, but the level of phosphorylation at steady state photosynthesis was not as great as observed on the first exposure to Light 1. It is evident in maize leaves that although the switches from Light 2 to Light 1 and vice versa produce the predicted dephosphorylation and phosphorylation, respectively, of LHC II, these changes in LHC II phosphorylation are not correlated either to changes in ϕ_{CO_2} or $\phi_{PS\,II}$.

Discussion

The data shown in Figs. 1, 2 and 3 demonstrate that transfer of wheat leaves from light exciting preferentially PS II to light exciting preferentially PS I, and vice versa, will induce a State transition that correlates with a change in the degree of phosphorylation of LHC II. However, the data for ϕ_{CO_2} indicate that such State transitions are not associated with changes in the photosynthetic apparatus that serve to optimise the efficiency of light use for CO_2 assimilation. The State transitions are accompanied by consistent changes in Fv'/Fm'; this parameter decreases during the transition from State 1 to State 2 and increases on transition from State 2 to State 1. Since Fv'/Fm' estimates the probability of excitation energy in the PS II antennae reaching an *open* PS II reaction centre, the changes in this parameter that occur during State transitions are consistent with changes in the antennae of PS II. The increase in Fv'/Fm' during a transition from State 2 to State 1 indicates that a modification is occurring in the PS II antennae that increases the probability of

excitation energy transfer to a reaction centre; a decreased probability of dissipation of excitation by non-radiative decay processes (non-photochemical quenching) could account for this. Modification of the PS II antennae by dephosphorylation of phosphorylated LHC II would be predicted to increase the absorption cross section of PS II. However, it would appear that dephosphorylation of LHC II must also modify the quenching characteristics of the antennae which result in a decrease in the probability of non-photochemical quenching. By the same argument LHC II phosphorylation on transition from State 1 to State 2 will decrease the absorption cross section of PS II and increase the probability of excitation decay by non-photochemical quenching. It is important to note that although consistent changes occur in Fv'/Fm' during State transitions, these changes do not always determine the quantum efficiency of PS II photochemistry. This is clearly seen during the switch of wheat leaves from Light 1 to Light 2 when Fv'/Fm' decreases but $\phi_{PS\,II}$ increases due to an increase in q_P (Fig. 2). In this situation decreasing the probability of excitation transfer to a reaction centre does not result in a decrease in electron flux through PS II reaction centres, presumably because non-cyclic electron flux is determined by factors down stream from the PS II reaction centre. The exact location of this regulation cannot be determined from this study, however the cytochrome b_6/f complex is an obvious candidate.

The experiments with the barley *chlorina* mutants demonstrate clearly that LHC II plays an important role in determining the ability of the leaf to perform a State transition. The absence of LHC II correlates with a loss of the ability of the leaves to modify Fv'/Fm' in response to switching between Light 2 and Light 1. However, it is evident that LHC II plays no role in determining the efficiency of light use for CO_2 assimilation since the changes in ϕ_{CO_2} of leaves of the mutants in response to switching between Light 1 and Light 2 are similar to those found in wild type leaves. Further evidence to suggest that LHC II phosphorylation does not play a role in optimising the quantum efficiency of CO_2 assimilation is found in maize leaves where phosphorylation and dephosphorylation of LHC II on switching between Light 1 and Light 2 does not correlate with changes in ϕ_{CO_2} (Figs. 3, 6). Clearly the change in light regime produces the expected change in the phosphorylation status of LHC II in the maize leaf, but surprisingly this is not accompanied by the changes in Fv'/Fm' that would be predicted in accordance with a phosphorylation-induced change in the PS II antennae. The regulation of photosynthetic carbon metabolism in maize is very different to that in C_3 plants and the presence of two very distinct populations of thylakoids in chloroplasts of the mesophyll and bundle sheath tissues would suggest that responses to changes in light regimes that preferentially excite PS I or PS II might well be very different compared to the response of thylakoids in C_3 leaves. However, it is surprising to find that LHC II phosphorylation does not appear to modify, in the predicted fashion, the effective antennae size of PS II since the bulk of PS II is in the mesophyll cells that have similar metabolic requirements to C_3 leaves for the stoichiometry of ATP:NADPH (3:2) production.

In conclusion, this study has demonstrated that (i) in C_3 leaves State transitions are associated with changes in the effective antennae size of PS II that correlate with changes in the phosphorylation of LHC II, although this is not the case in C_4 leaves of maize; (ii) State transitions do not serve to optimise the efficiency of the use of absorbed light for CO_2 assimilation in C_3 or C_4 leaves. It is not evident from this study what physiological significance State transitions have in C_3 leaves; this question requires further investigation.

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