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Regulation of Photosystem II by Metabolic and Environmental Factors [and Discussion]

P. Horton, J. Crofts, S. Gordon, K. Oxborough, D. Rees, J. D. Scholes, U. Schreiber and J.-M. Briantais

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Regulation of photosystem II by metabolic and environmental factors

By P. Horton, J. Crofts, S. Gordon, K. Oxborough, D. Rees and J. D. Scholes

Department of Biochemistry and Research Institute for Photosynthesis, University of Sheffield, Sheffield S10 2TN, U.K.

The thylakoid membranes of higher plants possess several mechanisms that control both the distribution and rate of dissipation of absorbed light. These mechanisms, which allow regulation of photosynthetic electron transport in response to alteration in external and internal factors, can be observed as the various processes that quench chlorophyll fluorescence. By using the 'light-doubling techniques', together with analysis of quenching relaxation, it is possible to assess quantitatively the extents of these regulatory processes and to allow their interrelations to be studied. These techniques can be applied to *in vitro* systems or to leaves, and can be particularly useful when applied with electron-transport measurements and when models are used to aid interpretation. Results of quenching analysis at different light intensities in isolated thylakoids, intact chloroplasts, protoplasts, algae and leaves of a variety of species are presented.

Introduction

The thylakoid membranes of plant chloroplasts experience large fluctuations in the intensity and spectral quality of absorbed radiation. There is genetic diversity and phenotypic plasticity in thylakoid composition, which is thereby adapted to the irradiance conditions in terms of the position in a canopy and within individual leaves. The composition of thylakoid membranes is dependent not only on the light environment but also on the balance established by other external and metabolic factors. The range of external conditions or 'window' in which photosynthesis can operate with maximum efficiency is thereby created. It has been suggested that short-term thylakoid regulation is responsible for, in effect, enlarging this window such that optimum efficiency of light harvesting is achieved in low light, but effective energy dissipation ensured in high light (Horton 1985*a*, *b*, 1986; Horton *et al.* 1988). The molecular processes involved in the regulation of light harvesting and electron transport are incompletely understood. Processes so far implicated in regulation include protein phosphorylation, which controls the relative absorption cross sections of PSI and PSII (Horton 1983*a*), ΔpH-induced change in radiationless decay of excitation energy (Briantais *et al.* 1979), photosystem II cycling (Oxborough & Horton 1988; Falkowski *et al.* 1988; Schreiber & Rienits 1986), and the light-activated violaxanthin cycle (Demmig *et al.* 1987).

Examination of chlorophyll fluorescence can provide a valuable insight into the processes that control the utilization and dissipation of excitation energy. Whereas photochemical quenching (q_Q) provides a measure of the fraction of excitation reaching open reaction centres of photosystem II (and is hence an indicator of changes in photochemical efficiency due to redox feedback from the electron-transfer chain), changes in non-photochemical quenching

(q_N) are indicative of regulation of the dissipation of excitation energy in the thylakoid. The complementary relation between q_Q and q_N first indicated that dissipation of excitation energy is increased as the proportion of excitation energy used photochemically is decreased (Horton 1986). The protective role of this quenching has been experimentally demonstrated (Krause & Behrend 1986). Techniques to resolve q_Q and q_N are based on the 'light-doubling' rationale conceived by Bradbury & Baker (1981), and developed in terms of modulated light by Quick & Horton (1984*b*). It became clear that q_N was heterogeneous and although the ΔpH-dependent q_E component is often the major one, protein phosphorylation-dependent state transitions (q_T) and changes associated with high-light treatment (q_I) can be large and important quenchers (Baker & Horton 1987). Recently it has been shown that q_E, q_T and q_I can be estimated on the basis of their different kinetics of relaxation upon cessation of illumination (Horton & Hague 1988).

Various aspects of chlorophyll fluorescence quenching are described below, and evidence for cycling of electrons around PSII as an important photochemical dissipative pathway is presented.

Materials and methods

Intact chloroplasts were isolated from 11–14-day-old pea plants (*Pisum sativum* L. cv. Kelvedon Wonder) as described by Oxborough *et al.* (1987) or from spinach according to Walker (1971). Protoplasts were prepared from barley (*Hordeum vulgare* L. cv. Marko) by the method of Quick & Horton (1984*a*). PSII particles were prepared by using the procedure of Berthold *et al.* (1981). Simultaneous measurement of O_2 evolution or uptake and chlorophyll fluorescence in chloroplast or protoplast samples used apparatus similar to that described by Horton (1983*b*) but modified to allow quantification of q_Q as described by Horton & Hague (1988). For leaf samples, a Hansatech LD2 electrode was used together with a Walz chlorophyll fluorometer. Chlorophyll fluorescence quenching coefficients q_Q (photochemical quenching), q_N (quenching by non-photochemical processes), q_E (energy-dependent quenching), q_T (state transition) and q_I (irreversible or photoinhibitory quenching) were calculated according to the principles of Schreiber *et al.* (1986) as described by Horton & Hague (1988).

Results and discussion

Regulation in low light

State transitions have been described in terms of chlorophyll fluorescence changes in conditions of extremely low light and in isolated thylakoids in the presence of ATP. Experiments with isolated protoplasts indicated a component of q_N that did not relax upon ΔpH elimination and which decayed with a half-time of *ca.* 8 min; this component was called q_T, and assigned to a state transition (Horton & Hague 1988). Figure 1 shows further support for the notion that this q_T component is indeed a measure of the transition to state 2 occurring upon illumination. Thus the relaxation is stimulated by far-red light, which would oxidize the plastoquinone (PQ) pool and decrease protein kinase activity. NaF, an inhibitor of protein phosphatase activity and of the transition to state 1 (Bennett 1980), inhibits the relaxation of this q_T component and eliminates the effect of far-red light. With this technique, it is therefore

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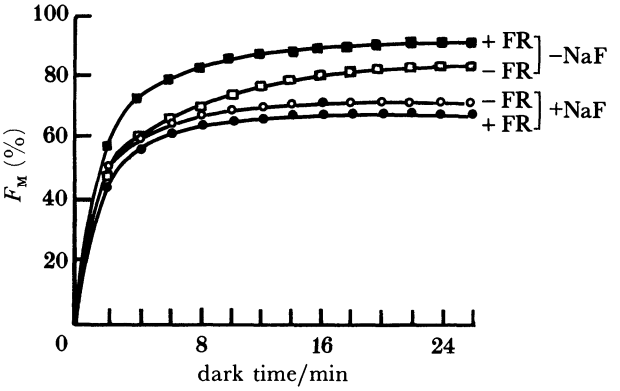

FIGURE 1. Relaxation of the F_M levels of fluorescence upon darkening of isolated protoplasts. Data points are values obtained upon pulsing every 2 min. NaF (50 mM) was added 30 s before darkening (○, ●). Far-red light was turned on immediately after the actinic light was turned off (■, ●).

possible to measure q_T at different light intensities; q_T reaches a maximum at low light intensity and thereafter decreases (figure 2). It is interesting that Q_A is maintained in a highly oxidized condition in this range of light intensity, suggesting that light-harvesting complex II (LHCII) phosphorylation has brought about optimal balancing of the photosystems. The appearance of q_T at low light intensities provides strong support for its role in enhancing photosynthetic efficiency by ensuring the correct ratio of excitation of PSII and PSI. The decline in q_T shown in figure 2 and by Horton & Hague (1988) is consistent with the documented control over protein phosphorylation by ΔpH (Fernyhough *et al.* 1984; Oxborough *et al.* 1987). Previous work with isolated chloroplasts had shown the complex interplay between redox state and energy state in determining the level of protein phosphorylation. This control mechanism is obviously dependent upon the provision of the appropriate stoichiometry of ATP and NADPH for metabolic processes in the plant (Horton 1985*a*, *b*). Experimental evidence supports the existence of an antiparallel relation between q_E and q_T. Recent data show that ΔpH alone is not sufficient for the suppression of protein phosphorylation; the development of q_E is necessary. Thus antimycin A, which eliminates q_E without decreasing ΔpH, can bring about a reduction in LHCII phosphorylation (Oxborough & Horton 1987; Oxborough *et al.* 1987).

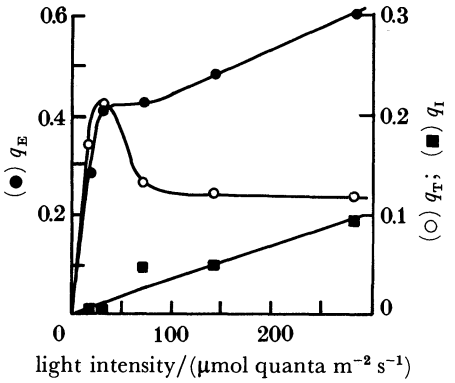

FIGURE 2. Assays of q_T (○), q_E (●) and q_I (■) in protoplasts illuminated with different light intensities (redrawn from Horton & Hague (1988)).

The close relation between q_T and photosynthesis is also shown by the data in figure 3. The re-establishment of the induction period for O_2 evolution strongly correlates with the relaxation kinetics of q_T. Similarly, the amount of O_2 evolved during each of the saturating pulses used to determine quenching relaxation also decays with the same kinetics as q_T. Consistent with this rationale is that NaF prevents the re-establishment of induction. Re-illumination in the presence of NaF induces multiple oscillations in the rate of O_2 evolution (figure 4). Although induction and oscillatory behaviour are complex phenomena involving a multitude of processes (Walker 1981) these observations point to an important role of the ATP/NADPH stoichiometry and its control by protein phosphorylation (Horton 1985*a*; Quick & Horton 1984*a*).

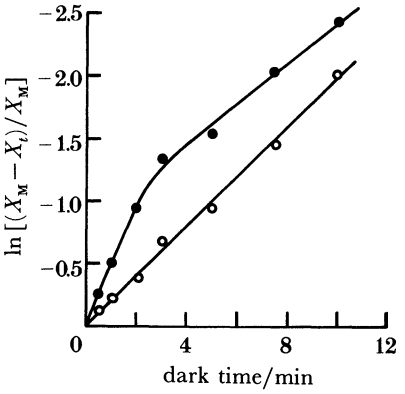

FIGURE 3. Semi-logarithmic plots of the relaxation of F_M (●) and the increase in length of the induction period for O_2 evolution (○) in isolated protoplasts. X_M and X_t refer to the fully dark-adapted maximum value (obtained before illumination) and that obtained t min after darkening.

Regulation in high light

In high light, or when metabolic processes are restricted, processes of dissipation indicated by q_{NP} are induced. The view is widely held that excessive excitation energy in the pigment bed can lead to photodamage. Included in this scenario is the loss of photosynthetic efficiency and capacity termed photoinhibition (Powles 1984; Osmond 1981). Photoinhibition is associated with loss of variable fluorescence and is included in the q_I term described above. There is still no agreement as to the mechanism of q_I, although it is likely to include processes that result from both damage or loss of PSII reaction-centre polypeptides and events in the chlorophyll antenna (Bradbury & Baker 1986) possibly associated with zeaxanthin formation (Demmig *et al.* 1987). It is clear from *in vitro* experiments that both q_T (Horton & Lee 1986) and q_E (Krause & Behrend 1986) can provide protection against photoinhibition. Figure 5 shows a titration of q_I with antimycin A. Low antimycin A removes q_E and is accompanied by increased q_I (this experiment used intact chloroplasts in the presence of saturating methyl viologen, precluding any q_T formation). Of interest is the fact that uncoupling at higher concentrations of antimycin A results in a further increase in q_I. This suggests that both q_E and ΔpH can bring about protection against photoinhibition. Experiments also show that q_I is associated with progressive reduction of the acceptor side of PSII; a negative correlation was found between q_Q and q_I (Horton *et al.* 1988), an observation consistent with the hypothesis that photoinhibition results from damage to the Q_B polypeptide (Kyle *et al.* 1985).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

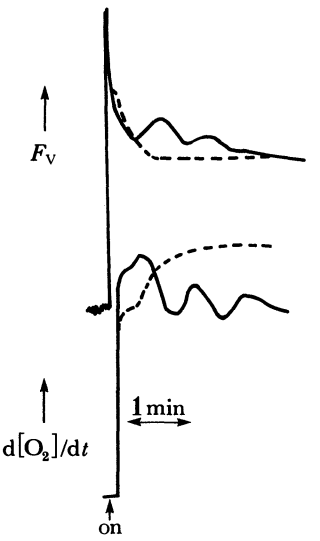

FIGURE 4. Effect of NaF on the kinetics of O_2 evolution and chlorophyll fluorescence. Protoplasts were illuminated for 5 min to reach steady state and then re-illuminated following a 30 s dark interval. 50 mM NaF was added 30 s before cessation of illumination (solid line).

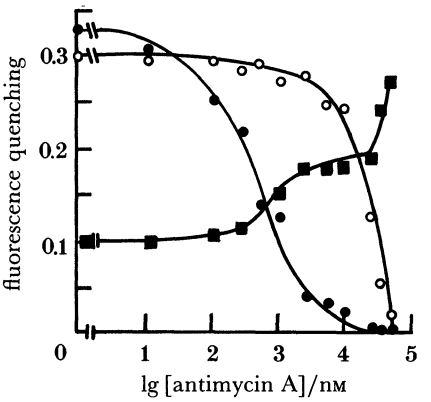

FIGURE 5. Titration of fluorescence parameters q_E (●) and q_I (■) with antimycin A. Isolated pea chloroplasts illuminated in the presence of methyl viologen for 8 min with 1080 μmol quanta m^{-2} s^{-1} at different antimycin A concentrations. Also shown is the quenching of 9-aminoacridine fluorescence (○) as an indicator of ΔpH (redrawn from Oxborough & Horton (1988)).

It is therefore of interest that even at relatively high light intensities q_Q remains large. This can be seen, for example, in figure 6 where a q_Q of 0.8 was observed at 75% saturation of photosynthesis. Protective mechanisms apparently keep Q_A oxidized. This phenomenon can be quantified by using the rationale of Weis & Berry (1987), who compared the measured quantum yield of electron transport, ϕ_s, and the fraction of excitation reaching open reaction centres, q_Q. In theory, this ratio should be constant, irrespective of the light intensity and the fraction of open centres. The ratio ϕ_s/q_Q is termed the intrinsic yield, ϕ_p, and is, in fact, found to decline as the light intensity increases (Weis & Berry 1987; Horton & Hague 1988; Horton *et al.* 1988). There are two explanations for this phenomenon, each of which defines alternative dissipative processes. Firstly, the dissipation of excitation energy indicated by q_E could significantly decrease that available for photosynthesis. This suggestion is based

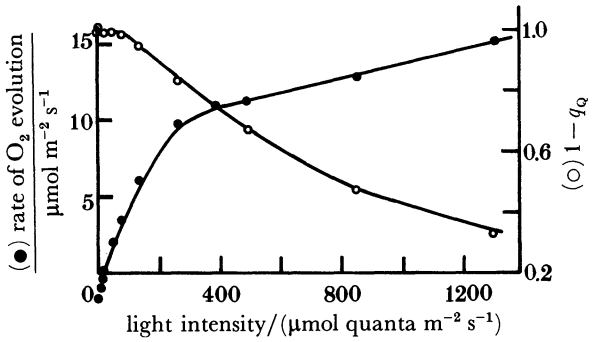

FIGURE 6. Rate of O_2 evolution (●) and q_Q (○) in spinach leaf discs at different light intensities. Parameters were measured after 4 min at each light intensity on the same leaf discs. q_Q was measured by a light pulse of 4750 μmol quanta m^{-2} s^{-1}. Each point is the mean of three measurements on separate leaves.

upon the observed correlation between q_N and ϕ_p. Alternatively, there could be photochemical dissipation by, for example, a PSII cycle. Isolated chloroplasts were used to try and distinguish these two possibilities. In figure 7, ϕ_s is plotted against q_Q at different light intensities. The nonlinear relation is indicative of the decline in ϕ_p at high light. Figure 8 indicates that antimycin A can remove q_E without affecting ϕ_p. Thus the decline in ϕ_p is not dependent upon

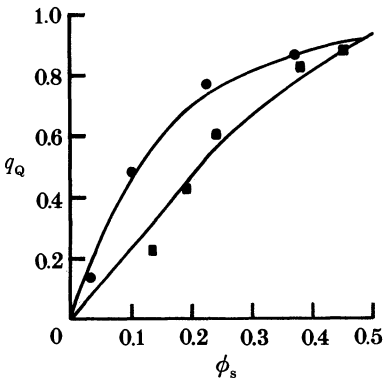

FIGURE 7. Quantum yield (ϕ_s) and q_Q in isolated spinach chloroplasts illuminated with light intensities between 50 and 3000 μmol quanta m^{-2} s^{-1} in the presence of methyl viologen. Control (●) and in the presence of 1 μM nigericin (■).

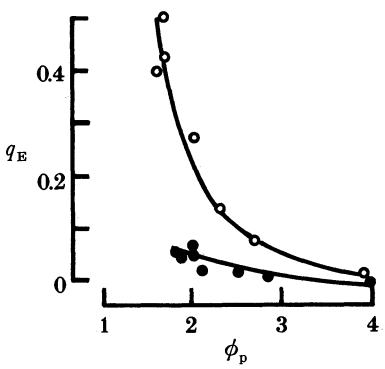

FIGURE 8. Relation between ϕ_p and q_E in isolated pea chloroplasts illuminated with a range of light intensities between 0 and 1080 μmol quanta m^{-2} s^{-1}. Control (○) and in the presence of 3 μM antimycin A (●) (redrawn from Oxborough & Horton (1988)).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

excitation-energy dissipation through q_E but is instead a result of another effect of high light. Addition of the uncoupler nigericin is able to increase ϕ_p at high light and to give rise to a linear ϕ_s:q_Q plot (figure 7). These data indicate that it is ΔpH controlling ϕ_p by a process involving photochemical dissipation. Two further experiments can separate q_E from ϕ_p. In the first, q_E and ϕ_p are titrated against ΔpH; q_E is found to require a larger ΔpH than the decrease in ϕ_p (figure 9). A second experiment took advantage of the observation that isolated spinach thylakoids often show negligible q_E even though a ΔpH is generated and photosynthetic control is evident. Thus addition of nigericin to coupled thylakoids resulted in a maximum increase in the rate of electron transport and q_Q within 20 s. No q_E was observed, yet there was a doubling of the calculated ϕ_p (figure 10).

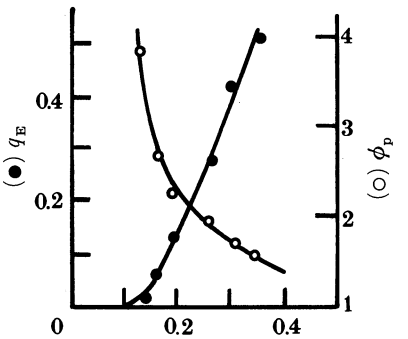

FIGURE 9. Dependency of q_E (●) and ϕ_p (○) upon ΔpH, measured with 9-aminoacridine fluorescence, and established by illumination of pea chloroplasts with different intensities.

It is suggested that ΔpH can induce a cycle around PSII and thus cause decrease in ϕ_p. One possibility is that it is the lumen pH, which is increasingly acidic in high light, that is modifying donor-side activity in PSII and so allowing a PSII cycle. To examine this hypothesis, isolated PSII particles have been subjected to illumination at low pH (figure 11). ϕ_p showed a sharp decline at pH < 6.5 such that at pH 5.0 it had fallen by 50%. Estimates of ΔpH in high light are in the range 2.5–3.0, with a stromal pH of 7.5–8.0, suggesting lumen pH is within the range of 4.5–5.5. Thus the 50–60% decrease in ϕ_p recorded *in vivo* is entirely consistent with the data in figure 11. Decreasing the pH also brought about a decrease in F_V/F_M of the PSII particles but the pH dependency is not the same; only below pH 5 is significant quenching of fluorescence observed. This suggests that q_E may also be caused by lumen acidification with larger ΔpH values being required for q_E formation compared with changes in ϕ_p (cf. figure 9). In this way the PSII cycle and q_E will act in concert to dissipate excitation both non-photochemically and photochemically in response to light-induced ΔpH formation.

Evidence has been obtained for PSII cycling in high light (Falkowski *et al.* 1988), upon donor side limitation on adding ADRY reagents (Heber *et al.* 1979) and upon ATP addition (Schreiber & Rienits 1986). The data described here are consistent with these reports and suggest that PSII cycling is an important mechanism for energy dissipation in high light. The pathway of this cycle is unknown but because q_Q as determined by light pulsing and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) addition are always very close (Quick & Horton 1984*b*) the electron must be flowing through the Q_B site. The change in redox potential of cytochrome b_{559} between pH 6 and 5 (Horton & Cramer 1975) and its photooxidation at low

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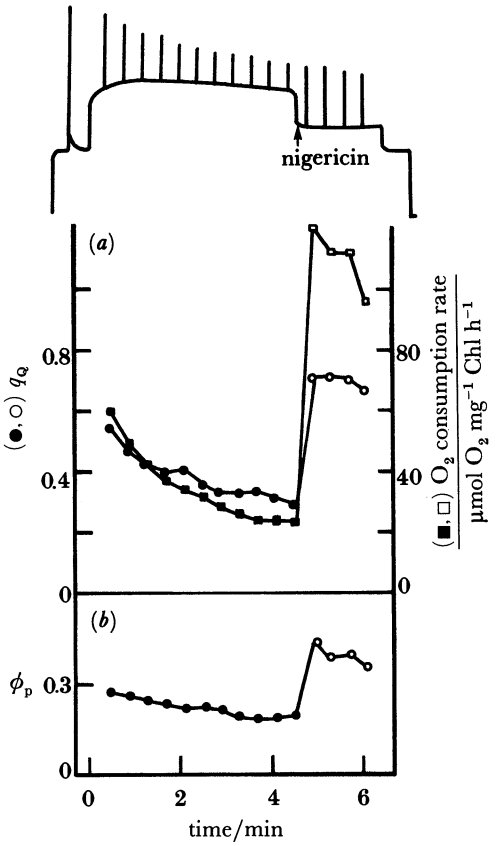

FIGURE 10. (*a*) Electron transfer and (*b*) chlorophyll fluorescence in spinach chloroplasts reducing methyl viologen. Nigericin (1 μM) was added as indicated. ϕ_p and q_Q were calculated by using light pulses as shown in the upper figure. Light intensity was 400 μmol quanta m^{-2} s^{-1}.

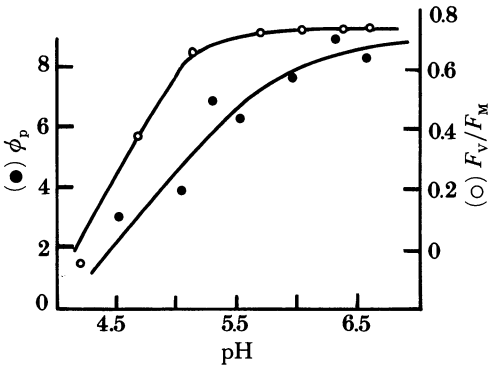

FIGURE 11. Effect of pH on the intrinsic yield of PSII, ϕ_p and F_V/F_M in isolated PSII particles. ϕ_p was measured by O_2 evolution in the presence of 2,6-dichlorobenzoquinone and ferricyanide at a light intensity of 1500 μmol quanta m^{-2} s^{-1}. q_Q was established with a single saturating pulse given during a constant rate of electron transport (after 2 mins). F_V/F_M was measured from F_O and F_M (using a light saturation pulse) in the absence of electron acceptor.

pH (Horton & Cramer 1976) suggest that this component is involved in the PSII cycle. Photooxidation of cytochrome b_{559} has been observed under a number of conditions when the donor side of PSII is perturbed (Cramer *et al.* 1979).

Conclusions

As plants proceed from darkness to full sunlight a successive series of events takes place in the thylakoid membrane. At low light, the light harvesting system adjusts to give optimal balance of PSII and PSI via reversible LHCII phosphorylation. At higher light, photochemical dissipation controls the redox state of PSII and prevents photoinhibition. Thermal dissipation of excitation energy as indicated by q_E is also a protective measure that will become increasingly important as light saturation is approached. The redox control of q_E in some way provides a link between the phosphorylation of LHCII and the level of thylakoid energization. At still higher light levels photoinhibition occurs; at least a part of this phenomenon is readily reversible and may itself be protective. The demands of metabolic processes for ATP and NADPH in the plant are important modulators of all these events, as are the dynamics of the changes in the light environment.

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Discussion

U. Schreiber (*Department of Botany, Universität Würzburg, F.R.G.*). As I said in my paper, I am in favour of the concept of cyclic PSII electron flow. In my opinion, it may well turn out that the $PSII_\varepsilon$ in Dr Weis's model corresponds to a population of cyclic PSII. Actually, the induction kinetics in saturating light show that the 'thermal' I_1–I_2 phase slows down in the same pH range (below pH 5) where Dr Horton observes formation of lower potential cytochrome b_{559}. It is important to realize that the cycling centres are transiently blocked during a pulse of saturating light. Hence, they will be considered 'open centres'. But obviously they cannot contribute to linear electron flow. This is analogous to the situation where cyclic photochemical quenching is induced by an ADRY reagent.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

J.-M. Briantais (*C.N.R.S.I.P.V.*, 91198 *Cedex*, *Gif sur Yvette*, *France*). Dr Horton showed that q_T associated with LHCII phosphorylation is maximum in very low light intensity where q_Q is still very high. Are plastoquinones then reduced enough to activate kinase because of a high value of the constant for the equilibrium: $Q_{A,red}\,PQ \overset{K}{\rightleftharpoons} Q_A\,PQ_{red}$?

P. Horton. It is indeed surprising that apparently maximum extents of q_T are seen under oxidized conditions. However, three points should be made: (1) the state transition will lead to balance between the photosystems and hence oxidation of the acceptor side of PSII, and the controlled state represents a balance between kinase activation, phosphatase activity and photon flux; (2) Q_A will be more oxidized than the intersystem carriers under low irradiance when redox equilibrium will be reached; and (3) it is still a matter of uncertainty as to what component is responsible for activating the protein kinase.

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