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Regulation of Photosystem II

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Introduction

The establishment of the redox nature of oxygenic photosynthesis, in which Robin Hill's demonstration of the 'Hill reaction' played such an important role, was one of the major advances in biology this century. His subsequent delineation of the 'Z' scheme had implications, the full extent of which are only beginning to be explored some 25 years later. One such implication is the requirement for regulation of the photosystems; not only does the Z scheme require that excitation of each photosystem has to be balanced for optimal quantum efficiency, but there has to be flexibility to accommodate changes in the light environment and in the metabolic requirement for the reducing power and the protonmotive force that are its direct products (Horton 1985). Moreover, the implied stoichiometric relationship between reaction centre contents and electron transfer components means that reaction centres have to be served by large light-harvesting systems if photosynthesis is to occur at a rate sufficient for autotrophic growth. These light-harvesting structures need to contain pigments organised in such a way as to promote efficient energy transfer to the reaction centre. The advantage this confers in limiting light is shown by the increase in ratio of light-harvesting pigments per reaction centre when plants (or in fact any photosynthetic organism) are grown in shade. The result of such adaptation is, however, that photosynthesis is saturated at relatively low light intensity. As a consequence, at high light intensity, the rate of absorption exceeds to a greater or lesser extent the rate at which it can be used photosynthetically. Under such conditions the photosynthetic system will be predisposed to light-induced damage, or photoinhibition (Osmond 1981). In oxygenic photosynthesis, it is clear that Photosystem II is the more susceptible to photoinhibition – this can be attributed to the facts that trap closure in PS II is associated with an increase in exciton density in the PS II antenna, the possibility of reactive radical species forming at the Q_B binding site (Kyle et al. 1985) and the highly oxidising P_{680}^+ which will be formed with higher frequency as irradiance increases (Cleland 1988). Photoinhibition has been associated with damage to the antenna and the reaction centre of PS II (Bradbury and Baker 1986). It would be expected, therefore, that there would exist regulatory mechanisms that are designed to protect Photosystem II from photoinhibition. The aim of this article is to review the current state of knowledge of such processes.

Evidence for regulation of Photosystem II

The first observation of regulation of PS II arose from studies of the state 1 to state 2 transitions (Bonaventura and Myers 1969), which were subsequently shown to depend on the phosphorylation of the PS II light harvesting complex, LHC II (Bennett et al. 1980). Experiments showed that phosphorylation of LHC II resulted

in a decrease in the PS II antenna size (Horton and Black 1981) and a reduction in the susceptibility to photoinhibition (Horton and Lee 1985). Such observations led to the development of the concept of feedback mechanisms to reduce the rate of excitation of PS II under conditions of high light (Horton 1985, 1987). However, it was clear that LHC II phosphorylation could at most reduce the antenna size by 25%, as quite easily seen from the maximum extent of the ATP-induced quenching of chlorophyll fluorescence (Horton and Black 1981). Attention was therefore focussed upon the role of high-energy state quenching or q_E – the non-photochemical quenching (q_N) of chlorophyll fluorescence that is induced in the presence of the thylakoid ΔpH (Briantais et al. 1979). With methods to separate photochemical quenching (q_P) from q_N (Bradbury and Baker 1981, Krause et al. 1982, Quick and Horton 1984) it was possible to show that at increasing light levels, the decrease in q_P was compensated by an increase in q_N – total quenching was independent of light intensity over a very wide range (Quick and Horton 1984, Horton and Hague 1987). The significance of this only became clear when it was demonstrated that prevention of q_E formation accelerated photoinhibition (Krause and Behrend 1986). This protection was shown to be largely due to quenching itself rather than the ΔpH that was inducing it. Thus, the decrease in fluorescence yield was due to a dissipation of energy that was protecting against photoinhibition. In fact, the idea of photoprotective energy dissipation was also suggested as a physiological function for a part of the fluorescence quenching accompanying photoinhibition (Demmig and Bjorkman 1987, Demmig et al. 1987); this type of quenching was also reversible but much more slowly than q_E (Demmig and Winter 1988, Walters and Horton 1991). The role of q_N in regulation of Photosystem II electron transport was however not established in this work – i.e., it was not shown that energy dissipation occurred at the expense of trapping by the reaction centre. Thus, a major step forward was made by Weis and Berry (1987) when it was shown that the intrinsic quantum efficiency of PS II declined reversibly in high light. Previously, it had been assumed that, as the light intensity increases, the decline in the quantum yield of PS II (and photosynthesis as a whole) was associated with (and indeed due to) closure of PS II centres because of accumulation of Q_A^-. Now, it was shown that, as the calculated quantum efficiency of photosynthesis (Φ_s) declined, the reaction centres were not closing proportionally. A strongly non-linear relationship was recorded between Φ_s and q_P not only from measurements of CO_2 fixation in leaves (Weis and Berry 1987, Genty et al. 1989), but also from electron transport measurements in protoplasts (Horton and Hague 1987), algae (Horton et al. 1989) and isolated thylakoids (Oxborough and Horton 1988, Weis and Lechtenberg 1989, Rees and Horton 1990).

Sites of regulation of PS II

The discrepancy between Φ_s and q_P could be explained in two ways. Firstly, there could be electron transport through PS II which was not giving rise to O_2 evolution or CO_2 fixation. Secondly, non-photochemical energy dissipation could be the cause, if this dissipation competed with excitation delivery to the PS II reaction centre. It has been shown directly that non-photochemical energy dissipation decreases the rate of Q_A reduction (Genty et al. 1990a). However, few other studies have sought to analytically distinguish between these possibilities. Instead, the correlations that have been observed between the ratio Φ_s/q_P and q_N have been used to implicate non-photochemical energy dissipation as the only factor controlling PS II. This has, in part, been driven by a desire to find a method for measuring leaf photosynthesis by chlorophyll fluorescence alone. This desire has been satisfied since a good linear relationship has been found between the product $q_P \times F_v/F_m$ and Φ_s measured by gas exchange (Genty et al. 1989). A linear relationship has also been reported, however, between Φ_s/q_P and $1 - q_N$ (Weis and Berry 1987, Weis and Lechtenberg 1989). One problem is that the mechanism of energy dissipation induced in high light is not known – surprisingly in fact, the existence of such correlations has been used as evidence for particular mechanisms for q_E.

Control of PS II by ΔpH

An important result was that uncoupling of isolated thylakoids lead to the observation of a perfect linear relationship between Φ_s and q_P (Weis and Lechtenberg 1989, Horton et al. 1989). Thus, it was the ΔpH that was the signal transducer in the regulation of PS II by light intensity. Since the major q_N component was the ΔpH-dependent q_E (Krause et al. 1982, Quick and Horton 1984) this was strong support for the involvement of non-photochemical energy dissipation in the control of PS II. However, the acidification of the thylakoid lumen is likely to have direct effects on the electron transfer reactions on the donor side of PS II and therefore it cannot be excluded that PS II is also regulated by this effect of the ΔpH. To test this suggestion we undertook a detailed series of experiments on isolated thylakoids. Firstly, a titration of Φ_s/q_P against ΔpH yielded a complex result, with two components; one occurred a ΔpH's less than that required form q_E, whilst the only the second coincided with q_E (Noctor and Horton 1990). Secondly, by using antimycin A to inhibit q_E without affecting ΔpH, it was possible to show significant ΔpH-dependent regulation of PS II without formation of q_E (Oxborough and Horton 1988, Rees and Horton 1990). Thirdly, by following the kinetics of Φ_s/q_P change upon illumination it was found that one component correlated with ΔpH formation whilst another accompanied the formation q_E, which develops more slowly than ΔpH (Rees and Horton 1990). These are clear demonstrations of direct ΔpH control over PS II electron transport in vitro that does not, in these cases, require q_E.

The study of ΔpH control of electron transport in isolated thylakoids was extended to consider the effects of low pH on PS II 'BBY' particles. It was found that lowering the pH to 5.0 resulted in a decline in Φ_s/q_P that was not accompanied by q_N (Crofts and Horton 1991). At pH 5 it was found that the quantum efficiency was reduced at low light intensity, similar to an ADRY-effect. The inhibition of electron donation at low pH has been reported many times (e.g. Wraight et al. 1972) and ADRY reagents have been shown to cause oxidation of Q_A (i.e. increase q_P). The best explanation for this effect of low pH is that it induces either an electron cycle around PS II or slow charge recombination within it. However, on the basis of the relationship between Φ_s and q_N in vivo, it has been concluded that this effect may be of minor physiological significance in the control of PS II during steady-state photosynthesis, although it may be important immediately following transitions in light intensity (Horton 1990).

High energy state quenching (q_E)

Evidence from gas exchange/fluorescence relationships

As mentioned above, the quenching of chlorophyll fluorescence associated with ΔpH formation is a long-established phenomenon. Early work indicated that this was an affect caused by acidification of the thylakoid lumen (Wraight et al. 1972, Briantais et al. 1979). Following the general formulation of fluorescence quenching introduced by Butler and co-workers, the principle question has been to consider whether q_E occurs in the PS II antenna or in the reaction center. One approach has been to examine the relationship between quenching and the associated effect on photochemistry, assuming that there is a complete causal link. This leads to the conclusion that neither sites of quenching can explain q_E; instead, it was proposed that q_E results from conversion of PS II from an active form into an inactive quenched state (Weis and Berry 1987). However, on the basis of an identical rationale and very similar experiments, the linear dependency of PS II efficiency on F_v/F_m was found to be fully consistent with quenching in the PS II antenna (Genty et al. 1989). As discussed above, it is dangerous to try to conclude too much about the mechanism of q_E from such experiments since gas exchange/fluorescence relationships can be affected by alterations in electron transport pathways as well as by artefacts resulting from factors such as leaf inhomogeneity. Despite this limitation it is clear that these experiments have been important in showing that non-photochemical energy dissipation is sufficient to explain control of PS II in vivo.

Studies on fluorescence quenching properties of q_E

Rather than looking at the effect of q_E on photochemistry, analysis of the characteristics of quenching itself has been undertaken. As first described by Bilger and Schreiber (1986), q_N is associated with quenching of F_0. Subsequent work confirmed the association of F_0 quenching with q_E (Rees et al. 1990, Genty et al. 1990b, Bilger and Bjorkman 1990, Gilmore and Yamamoto 1991). In a detailed analyses of leaves, algae and isolated thylakoids the relationship between quenching of F_0 and F_m were determined for q_E (Rees et al. 1990). The data fitted that predicted for antenna quenching in the Butler formulation. Furthermore, the observed $F_0:F_m$ relationship was identical to that observed for addition of artificial chlorophyll fluorescence quenchers, DNB and DBMIB. Thus, a simple model in which quenching centres are created in the PS II antenna, is a sufficient explanation of q_E. Similar conclusions had been reached earlier to account for the mechanism of the more slowly relaxing q_N associated with photoinhibition (Demmig and Bjorkman 1987).

Relationship between q_E *and zeaxanthin*

The slowly relaxing component of q_N induced under high light conditions was found to correlate with the extent of the enzymatic de-epoxidation of violaxanthin to zeaxanthin (Demmig et al. 1987). Since xanthophylls are associated with light-harvesting complexes rather than reaction centres and because of the observed quenching of F_0, it was concluded that this is a process occurring in the PS II antenna. In the course of these experiments it was noticed that leaves containing zeaxanthin showed more rapid formation of q_E (Demmig-Adams et al. 1989b). An extensive series of experiments showed that a linear relationship could be observed between the zeaxanthin content and the total thermal dissipation rate calculated from fluorescence quenching (Demmig-Adams et al. 1989a, Bilger and Bjorkman 1990). Remarkably, the data suggested that zeaxanthin was behaving as in ideal Stern-Volmer quencher in these experiments (Demmig-Adams 1990). The same conclusion was reached by Gilmore and Yamamoto (1991) following experiments on zeaxanthin formation in isolated chloroplasts. It was also found that inhibition of zeaxanthin formation with DTT inhibited q_E formation (Bilger and Bjorkman 1990, Demmig-Adams et al. 1990, Gilmore and Yamamoto 1991). However, q_E was not completely blocked, the proportion of q_E that is formed in the presence of DTT being variable. Such data led Demmig-Adams and co-workers to suggest that there are two types of q_E: one, a zeaxanthin dependent Stern-Volmer quenching in the PS II antenna and a second, due to a process in the PS II reaction centre (Demmig-Adams et al. 1990). The former type has been recently discussed at length by Demmig-Adams (1990), although the major problem remains the lack of a plausible quenching mechanism (an alternative explanation of the role of zeaxanthin is presented below). There is considerable precedent for the idea of a quencher in the PS II reaction centre and this is dealt with in the next section.

Quenching in the PS II reaction centre

It is well known that inhibition of electron donation by treatment with tris or NH_2OH or after oxidation by ferricyanide prior to coding to 77 K results in quenching. The quenching species is proposed to be $P_{680}{}^+$, analogous to the quenching ability of $P_{700}{}^+$, but this has not be experimentally proven. Alternatively, it is known that a chlorophyll in the reaction centre can become oxidised by tyr_Z when water oxidation is blocked (Thompson and Brudwig 1988); this oxidised species could be a quencher. Finally, there is evidence that a low pH inhibition of the donor side can induce rapid charge recombination within the PS II reaction centre; this could prevent trap closure during a saturation pulse and would therefore be perceived an non-photochemical quenching (Schreiber and Neubauer 1990). Therefore, in theory, a low pH inhibition of the PS II donor side provides a feasible mechanism for q_E. In support of this, acidification of thylakoids and PS II particles leads to extensive quenching (Crofts and Horton 1991) that can be eliminated if a reducing agent is added, to overcome the inhibition of the natural donation re-

action. However, detailed inspection suggests that this strongly redox-dependant pH quenching may not be the major process underlying q_E (Rees et al. 1992), although it cannot be discounted as a minor component. Thus, q_E in vivo results in up to 50% quenching of F_0, whereas 'donor side' quenching does not affect F_0. Also, the strict redox dependency of PS II quenching is not observed for ΔpH-dependent q_E; ascorbate, which abolishes donor side quenching, does not inhibit q_E. However, more work is still required to assess the physiological importance of what is clearly a *possible* consequence of lumen acidification.

Light-activation of q_E

Exploring the role of zeaxanthin in q_E in vivo is complicated by the fact that the influence of other factors cannot be easily assessed. To overcome this problem we have undertaken detailed analyses of chloroplasts isolated from leaves that have been pretreated so as to alter the zeaxanthin:violaxanthin ratios. The data provided no evidence to support the suggestions either that zeaxanthin was required for q_E or that there are two types of q_E. However, in this study, light-activation of q_E was discovered; hence, it was found that chloroplasts from light-treated leaves required a lower ΔpH to form q_E than dark control leaves (Rees et al. 1989, Noctor et al. 1991). The estimated lumen pH to give half maximal q_E was about 4.0–4.5 in 'dark' chloroplasts, increasing to 4.5–5.0 after light treatment. Recently, this activation was observed for pH-dependent quenching where this pH shift could be directly determined to be from 4.4 to 5.7 (Rees et al. 1992). Light activation correlated with zeaxanthin formation: on the basis of this data, it was proposed that zeaxanthin may be an activator of q_E. In line with analogies of activators of enzymes, it was suggested that zeaxanthin may not play a direct part in the mechanism of energy dissipation but may exert a 'structural' effect on a membrane protein complex. It should be pointed out that such a structural role does not exclude participation in a possible interaction with chlorophyll in the formation of the 'quenching' pigment species.

Identification of the site of q_E

A number of recent observations suggest that q_E results form the dissipation of energy in the light-harvesting complexes of PS II.

1. 77 K fluorescence. 77 K fluorescence emission spectra of chloroplasts frozen after induction of q_E indicated preferential quenching at 680 nm (Ruban et al. 1991). This is an emission band of LHC II rather than the PS II core. Furthermore, after light activation, this band shifted to 700 nm – again this emission is characteristic of LHC II. Significantly, F700 is observed when LHC II is aggregated, suggesting that light-activation results from a change in organisation of the LHC II complexes under the influence of zeaxanthin (Horton et al. 1991).

2. Absorbance changes. It has been known for many years that q_E was associated with changes in light scattering – in general these changes are detected in the spectral region 510–550 nm and were attributed to large scale changes in chloroplast volume that can accompany ΔpH formation (e.g. Deamer et al. 1967). Studies on the kinetics of light-scattering changes show them to closely match q_E (Sivak et al. 1985, Bilger et al. 1988). Recently, it was shown that if antimycin A is used to inhibit q_E formation, a major component of light scattering at 530 nm was inhibited (Horton et al. 1991) – this linked ΔA_{530} to q_E rather than ΔpH per se. Moreover, it indicated that q_E resulted from organisational changes in the thylakoid membrane, as first suggested by Krause (1973), rather than specific inhibition of PS II photochemistry. The exact origin of ΔA_{530} remains to be established, however. In experiments on leaves, ΔA_{530} was linearly related to $\Delta F_m/F'_m$, behaving as a perfect Stern-Volmer quencher (Ruban and Horton, unpublished). Similar absorbance changes are seen upon aggregation of LHC II in vitro (Ruban and Horton 1992).

3. Effects of antimycin A. The inhibitory effect of antimycin A on q_E was always hard to explain in terms of the action of an electron transport inhibitor (Oxborough and Horton 1987). The fact that it inhibited PS I cyclic electron transport

in a way unconnected to its inhibitory action of the mitochondrial bc_1 complex was also hard to explain. It suggested that the actions of antimycin A on chloroplast reactions may be a more general effect on the membrane, thus explaining how it could inhibit PS I and PS II associated processes. Antimycin A perhaps exerts a direct effect on the organisation of certain membrane proteins, explaining its effect on q_E. Thus, the aggregation of detergent-solubilised LHC II that is induced by dialysis against a detergent-free medium, can be inhibited by antimycin A with an I_{50} of 200 nM, very similar to the concentration required to inhibit q_E (Horton et al. 1991, Ruban et al. 1991a).

4. *LHC II deficient mutants*. It has been shown that q_N is much smaller in the chlorophyll *b*-less *chlorina f*2 mutant of barley and that the control of PS II photochemistry by q_N is also poor in the mutant (Genty et al. 1990c).

Mechanism of q_E

From the above it can be seen that there are probably two synergistic factors driving the formation of q_E – the acidification of the thylakoid lumen and the enzymatic conversion of violaxanthin to zeaxanthin. This leads to the proposal that q_E involves transitions between discrete conformational states of the PS II light-harvesting system (Horton 1989), in line with the established principles for regulation of enzyme activity. The evidence indicating that the principal site of energy dissipation is in LHC II and that there is a link between q_E and LHC II aggregation enabled a new model for q_E to be devised (Horton et al. 1991). In this model there are a minimum of 4 states of the light-harvesting system which differ in the zeaxanthin/violaxanthin ratio and protonation/deprotonation of lumen-exposed residue(s) (Fig. 1): (I) Unprotonated, zero zeaxanthin, highest efficiency of transfer to PS II; (II) Protonated, zero zeaxanthin, low transfer efficiency; (III) Unprotonated, zeaxanthin enriched complex, slightly reduced efficiency; (IV) Protonated, zeaxanthin complex, low transfer efficiency.

In the experiments described above, chloroplasts were prepared in forms (I) or (III) by appropriate pre-illumination of leaves. The transition from I to II occurs with a p*Ka* of about 4.5 whereas for III to IV it increases to 5.7. We suggest that quenching results from changes in the interaction between LHC II trimers by a mechanism that is simulated by aggregation of LHC II in vitro. Although the mechanism of fluorescence quenching occurring upon aggregation of LHC II is not established, a number of changes in the absorption spectrum occur that indicate increases in pigment interaction; in particular, a long wavelength chlorophyll species

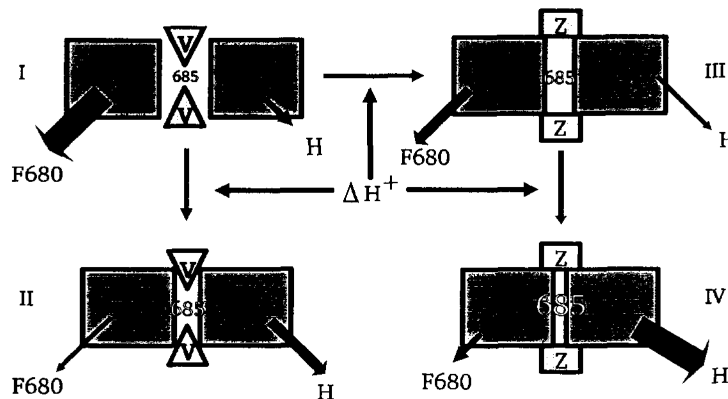

Fig. 1. LHC II model for q_E. Shown are 4 states of the LHC II complexes, I, II, III and IV. Fluorescence emission is indicated by 'F680', chlorophyll aggregation by '685' and non-radiative energy dissipation by 'H'. 'z' and 'v' refer to zeaxanthin and violaxanthin, respectively. Although the protonation of LHC II and zeaxanthin-associated LHC II structural change are formally presented here as separate processes, in effect, they should be viewed as synergistically driving the formation of the aggregated quenched form of the complex. For detailed explanation see text.

appears absorbing at 685 nm and emitting at 700 nm (Ruban and Horton 1992). It is probably this species that appears following light activation (Ruban et al. 1991). The strong correlative evidence for the role of zeaxanthin in q_E and the transfer of energy from a 505 nm band to the 700 nm emitting species leads us to suggest that the quenched state may involve a specific carotenoid/chlorophyll interaction (Ruban and Horton 1992).

The way in which low pH acts as the primary trigger for quenching remains to be established. The lumen facing domains of LHC II have a number of glutamate and aspartate residues (Karlin-Neuman et al. 1985); these could become protonated upon lumen acidification. Recently, it has been shown that some of these residues, located in a hydrophobic domain, have a role in H^+ transfer from the site of the O_2 evolution to the lumenal bulk phase (Jahns and Junge 1990); covalent modification with DCCD (dicyclohexylcarbodiimide), a reagent that binds to carboxyl groups in hydrophobic domains, resulted in inhibition of PS II-dependent lumen acidification. We have found that DCCD causes a decrease in the steady-state ΔpH and the inhibition of q_E (Ruban et al. 1992b); of particular interest is that DCCD also inhibits pH-dependent quenching, suggesting that these sites are not only involved in H^+ transmission but are directly involved in q_E. DCCD labels a number of LHC II polypeptides. Hence, we suggest that the primary event in q_E is the protonation of key glutamate/aspartate residues within the LHC II complexes, possibly those on CP 29 or CP 24 which are located close to the PS II core; this will cause a conformational change in the LHC II polypeptide affecting the environment of the densely packed chlorophyll molecules. A series of long range transitions within the LHC II complexes also follow, giving rise to the macroscopic structural changes associated with q_E; it is these structural changes in LHC II that are inhibited by antimycin A. The fact that apparent pK*a* of these residues would appear to be changed following light activation suggests tight coupling between protonation and LHC II conformation. This is also supported by the fact that when q_E is inhibited by antimycin A the apparent pK*a* shifted to higher pH. The observation that the DCCD sites on LHC II are also Ca^{2+}-binding sites (Webber and Gray 1989) suggests some role for Ca^{2+} in q_E. In fact, Ca^{2+} channel inhibitors have been reported to block q_E formation (Weis, personal communication).

Physiological aspects of q_E

The LHC II model for q_E has numerous physiological implications. Explanation of the fluorescence induction kinetics in whole leaves can be found by application of this model. The kinetics of induction of non-photochemical quenching during the initial illumination of a spinach leaf has two phases (Fig. 2): the first phase reaches a plateau after 2 min illumination. We suggest this represents the transition from I to II in Fig. 1. After 4 min, a second phase of quenching begins and this continues during the second illumination period. At the end of this second period we

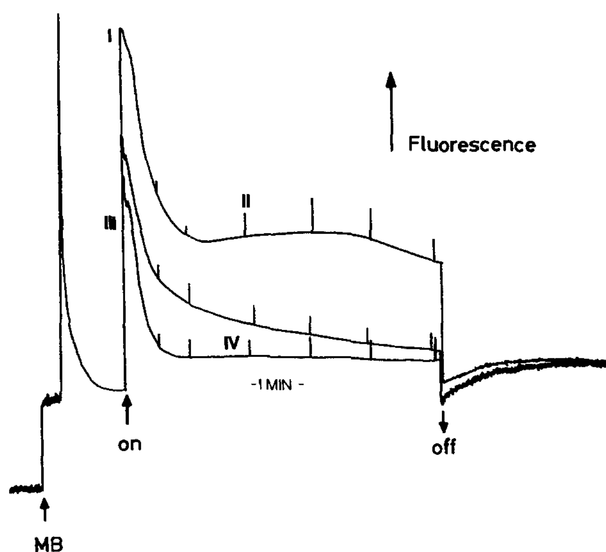

Fig. 2. Chlorophyll fluorescence changes in a dark-adapted spinach leaf. A spinach leaf was illuminated in air at an intensity of 800 μmoles m^{-2} s^{-1} for 3 successive 5 min periods separated by 3 min dark intervals. Chlorophyll fluorescence was measured with a Walz PAM chlorophyll fluorimeter. Saturating light pulses were given at 1 min intervals to measure the extent of q_P and q_N. I, II, III and IV refer to the postulated state of the LHC II at particular stages of induction of quenching. 'mb' refers to switching on the measuring beam and 'on' and 'off' to the start and end of the illumination period.

suggest that the system is at IV, relaxing to III in the following dark period; the third illumination now causes rapid quenching to the low fluorescence level (III to IV). The leaf has now been activated and all subsequent periods of illumination and brief dark adaptation involve only changes between III and IV. This explanation is consistent with the different q_E spectra for I to II and III to IV and with the kinetics of zeaxanthin formation (data not shown). If DTT is used to block zeaxanthin formation only the first I to II transition is observed. This data supports the notion that light activation of q_E is a significant process in vivo; we have previously suggested that the purpose is to allow q_E formation to occur at the sub-saturating ΔpH levels that are consistent with allowing high rates of electron transport. In vivo, it is unlikely that ΔpH will change over a sufficiently wide range to give maximum q_E unless there is reversible light-activation. It should be pointed out that ΔpH has not been measured in vivo, although indirect estimates based on the kinetics of re-reduction of P_{700}^+ suggest steady-state values are relatively low even in high light (Harbinson and Hedley 1992).

It is clear from a survey of the large number of papers describing non-photochemical quenching that the kinetics and extent are variable between species. A survey of a range of UK species indicates a factor of 2 difference in the maximum capacity for q_E, the capacity correlating with an ecological indicator of frequency at which each species is found naturally in open habitats (Johnson 1992). Even more extreme quenching is seen in plant species that are resilient to severe environmental stress. For example, the tropical epiphyte, *Guzmania monostacia* shows a 50% quenching of F_0 compared to about 25% in a spinach leaf (Fig. 3). Using the parameter ΔF_m/Fm′, a linear indicator of energy dissipation, whereas a spinach leaf at light saturation has a value of about 1.5, the value for *Guzmania* is approx 6.0. In such species the transition between I and III appears to occur more rapidly, such that quenching kinetics mainly reflect the transition III to IV. Consistent with this idea, DTT treatment of *Guzmania* inhibits most of q_E – i.e., there is negligible I to II transition. In other species that we have examined, quenching

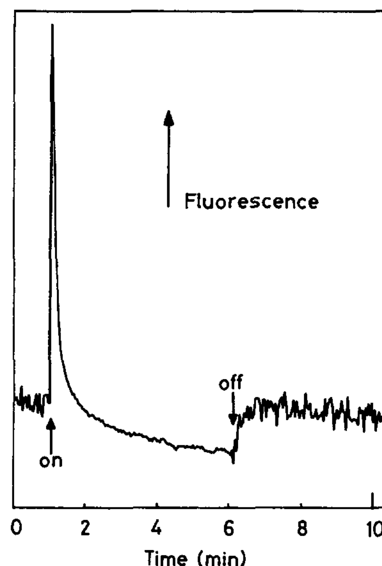

Fig. 3. Chlorophyll fluorescence changes in a dark-adapted leaf of *Guzmania monostacia*. Illumination was given at an intensity of 300 μmoles m^{-2} s^{-1} in air; under these conditions q_P was zero. The measuring beam was turned on at zero time.

appears to comprise a mixture of I to II and III to IV occurring roughly simultaneously. In *Guzmania*, photosynthetic capacity is very low (Maxwell and Griffiths, personal communication) and consequently it is likely that the capacity to generate high ΔpH is limited. Hence, formation of q_E will be very dependent on light activation. Consistent with this argument is the presence of high concentrations of xanthophyll cycle pigments in *Guzmania* and the large capacity and rapid kinetics of zeaxanthin formation (Young, personal communication).

In conclusion, it is clear that the characteristics of q_E are not only variable between species but that this variability appears to reflect adaptation to particular environmental conditions. It is not known what molecular features determine the capacity for energy dissipation. In terms of our model, a key feature will be the tendency of LHC II to form quenching centres; this will depend on a variety of factors including LHC II concentration and composition, xanthophyll composition, composition of other boundary lipids, ionic environment and polypeptide composition.

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