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# Allosteric regulation of the light-harvesting system of photosystem II

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Non-photochemical quenching of chlorophyll fluorescence (NPQ) is symptomatic of the regulation of energy dissipation by the light-harvesting antenna of photosystem II (PS II). The kinetics of NPQ in both leaves and isolated chloroplasts are determined by the transthylakoid  $\Delta\text{pH}$  and the de-epoxidation state of the xanthophyll cycle. In order to understand the mechanism and regulation of NPQ we have adopted the approaches commonly used in the study of enzyme-catalysed reactions. Steady-state measurements suggest allosteric regulation of NPQ, involving control by the xanthophyll cycle carotenoids of a protonation-dependent conformational change that transforms the PS II antenna from an unquenched to a quenched state. The features of this model were confirmed using isolated light-harvesting proteins. Analysis of the rate of induction of quenching both *in vitro* and *in vivo* indicated a bimolecular second-order reaction; it is suggested that quenching arises from the reaction between two fluorescent domains, possibly within a single protein subunit. A universal model for this transition is presented based on simple thermodynamic principles governing reaction kinetics.

**Keywords:** light harvesting; xanthophyll cycle; non-photochemical quenching; photosynthesis; chloroplast

## 1. INTRODUCTION

The flow of energy from the light-harvesting pigments to the reaction centre of photosystem II in plants is regulated (Horton 1985). Regulation appears to be necessary to adjust the photosynthetic system to large changes in irradiance—in limiting light, efficiency capture of excitation is promoted by efficient energy transfer from the light-harvesting complexes to the reaction centre. When irradiance exceeds the capacity for its use in electron transport there is an excess that continues to get larger with increasing rate of light input. Regulation is achieved by the conversion of the excess energy into heat (Horton *et al.* 1996). Excitation energy is quenched and this process is readily observed by the resultant decrease in fluorescence yield—hence the term ‘fluorescence quenching’. Because the quenching is not due to energy being used photochemically, it is termed non-photochemical quenching ( $q_N$  or NPQ). The major process giving rise to  $q_N$  depends on the energization of the thylakoid (the formation of the  $\Delta\text{pH}$ ) and is hence referred to as  $q_E$ . The reduction in lifetime of excited states that gives rise to  $q_E$ , the resulting decreased rate of excitation of the reaction centre of photosystem II (PS II) and the decreased reduction state of PS II are all considered to provide protection of the pigments and proteins of the photosystem from photodamage.

## 2. DEFINITION OF MECHANISM

The prime objective of research into this important regulatory process is the determination of its mechanism.

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The search to achieve this objective has been controversial and in part this is due to confusion over the definition of what is meant by the term ‘mechanism’. The mechanism of this process includes (i) where it *occurs*; (ii) how it is *induced*; (iii) how it is *controlled*; and (iv) how electronic excitation energy is *dissipated*. There has been a frequent failure to distinguish between these aspects in the formulation of hypotheses and in the design and interpretation of experimental data. Nearly ten years ago we proposed a hypothesis that clearly delineated these different aspects of mechanism (Horton *et al.* 1991), suggesting that

- (i) energy dissipation *occurred* in one or more of the proteins that constituted the light-harvesting system of photosystem II (LHC II);
- (ii) it was *induced* by a conformational change in one or more of these proteins;
- (iii) it was *controlled* by the synergistic effects of protonation of key amino-acid residues on these proteins and de-epoxidation of the carotenoid violaxanthin via the xanthophyll cycle;
- (iv) energy was *dissipated* because excitation energy absorbed by LHC II was converted into heat as a result of an altered chlorophyll–chlorophyll interaction in the system, which is known from model systems to decrease strongly the chlorophyll *a* excited state lifetime.

Furthermore, the rationale followed the ‘rules’ of enzymology,  $q_E$  being considered in the same terms as any regulatory enzyme-catalysed reaction. How an enzyme is regulated and the details of its catalytic mechanism can be viewed to be two quite separate aspects of its function. Indeed, it is possible to understand

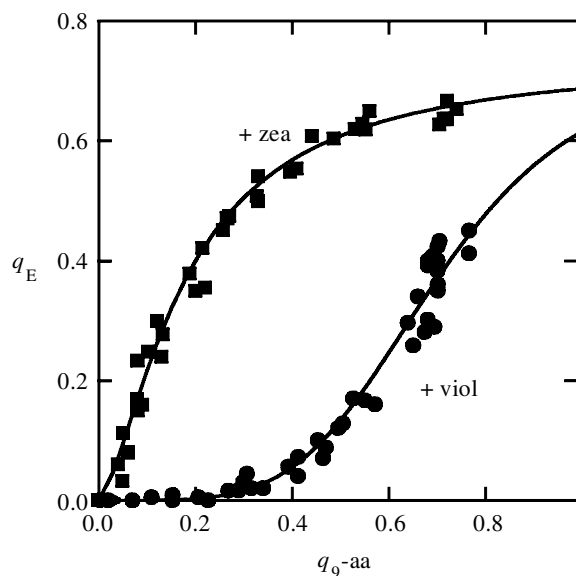


Figure 1. Titration of  $q_E$  against  $\Delta pH$  in isolated spinach chloroplasts.  $\Delta pH$  is expressed as the quenching of 9-amino-acridine fluorescence and  $q_E$  as the  $N$ -(3,4-dichlorophenyl)- $N'$ -dimethylurea-reversible quenching expressed as  $(F_m' - F_m)/F_m$ . Titration was obtained by varying the irradiance. Chloroplasts were prepared from dark-adapted leaves (+ viol) and light-treated leaves (+ zea). The experiment was carried out essentially as described by Noctor *et al.* (1991). The estimated pK values are 5.7 for light and 4.7 for dark.

fully the regulation of an enzyme without knowing the actual events occurring in its active site. In the case of  $q_E$ , it is perfectly legitimate to explore the events associated with its regulation without knowing exactly how the excited states are dissipated.

### 3. PROPERTIES OF ENERGY-DEPENDENT QUENCHING

Over the last ten years a great deal more about  $q_E$  has been learned. It has been confirmed as a process taking place in the light-harvesting proteins (Ruban & Horton 1994). Possible sites of interaction between protons and these proteins have been discovered (Walters *et al.* 1996; Pesaresi *et al.* 1997) and a role of the xanthophyll cycle has been established (Demmig-Adams *et al.* 1996). Evidence for a conformational difference between the quenched and unquenched state has been obtained (Ruban *et al.* 1993; Bilger & Björkman 1994), and the essential quantitative features of the control by proton and xanthophyll concentration have been described (Noctor *et al.* 1991; Gilmore & Yamamoto 1992; Gilmore *et al.* 1998). An *in vitro* model has been developed in which energy dissipation can be regulated in a manner that shares many of the same features as the process occurring *in vivo* (Ruban *et al.* 1994, 1996; Wentworth *et al.* 2000).

The part played by the xanthophyll cycle has been the most controversial. A direct role of the de-epoxidized carotenoids zeaxanthin and antheraxanthin as quenchers of singlet excited Chl has been repeatedly proposed (e.g. Demmig-Adams 1990), based on correlative data and

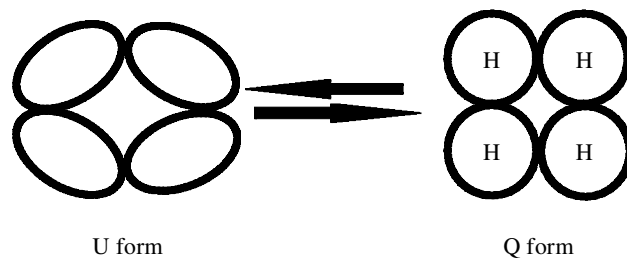


Figure 2. Allosteric model for the regulation of light harvesting. The transition between two states, one inactive in quenching (U form) and the other active (Q form). The important feature of the transition is the change in conformation of the monomeric subunit associated with protonation. This conformation is linked to a new oligomeric organization. Cooperativity of proton binding arises through the strength of the subunit-subunit interactions. Neither the identity of the protein subunits or the number of interacting subunits is known.

supported by theoretical estimates of the excited-state energy levels of violaxanthin and zeaxanthin (Owens *et al.* 1992; Frank *et al.* 1994). The fact that the correlation was frequently broken (e.g. Noctor *et al.* 1991) was initially ignored, and later attributed to the participation of another xanthophyll (lutein) not involved in the cycle. This proposition is quite contrary to the apparent absolute dependency of  $q_E$  on zeaxanthin and antheraxanthin seen in some thylakoid experiments.

Moreover, recent data show that the suggestion that the difference in energy levels was large enough to explain differences in quenching strength can be disputed (Polívka *et al.* 1999). In contrast, the proposition that the xanthophyll cycle carotenoids control the operation of an intrinsic quenching process in LHC II has received experimental support both *in vivo* and *in vitro* (Horton *et al.* 1996; Noctor *et al.* 1991, 1993; Ruban *et al.* 1994, 1996; Ruban & Horton 1999).

More recently, a genetic approach has been applied to this field (Horton 2000): mutants with deficiency in  $q_E$  have been selected; mutants with altered chlorophyll and xanthophyll composition have been analysed; and anti-sense expression has been used to manipulate the levels of proteins and pigments of the light-harvesting system. Whilst the analysis of these mutants and transgenic plants has not led to any firm conclusions about either the mechanism of control or the pathway of energy dissipation, new and unexpected facets that have to be explained have been uncovered (see §5). For example, the reduction in  $q_E$  in mutants lacking lutein has again pointed to a role of this carotenoid (Pogson *et al.* 1998). The *npq4* mutant of *Arabidopsis thaliana* is the most intriguing mutant (Li *et al.* 2000)—in leaves it completely lacks the rapidly relaxing  $q_E$  type of NPQ, yet it retains an active xanthophyll cycle and forms a  $\Delta pH$  (as assessed by its unchanged photosynthetic characteristics). It contains an apparently normal complement of Lhcb proteins, but instead lacks the LHC-related protein, PsbS. Therefore, it has been suggested that PsbS is the site of  $q_E$ , although an alternative explanation of the *npq4* phenotype is that PsbS may in some way regulate a quenching process elsewhere in the LHC II system (see §12).

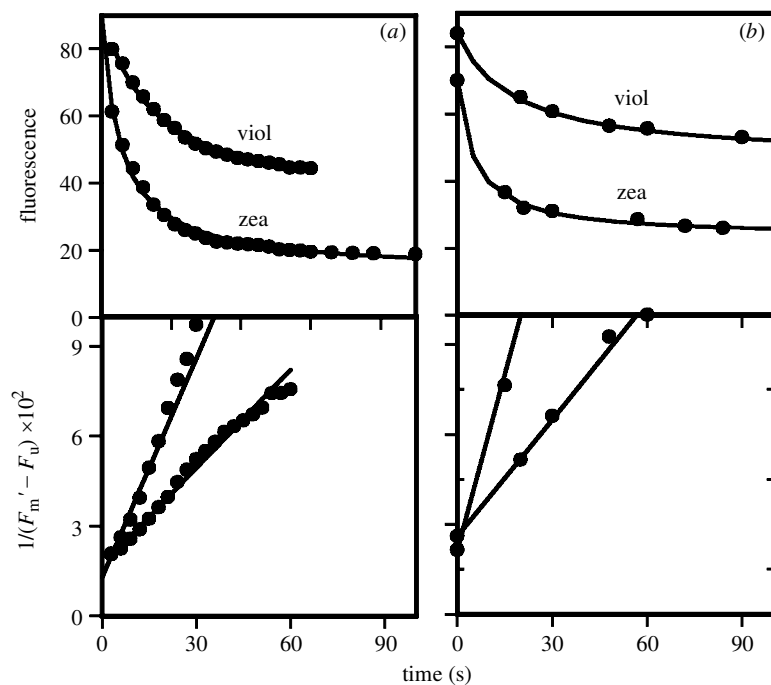


Figure 3. Kinetics of quenching in (a) spinach leaves and (b) chloroplasts. Top curves show the  $F'_m$ -values following illumination at time 0 with light saturating for  $q_E$  for dark-adapted (viol) and light-treated (zea) leaves. In the bottom curves the data are plotted to show that it is second order.  $F'_m$  is the  $F_m$  at the point following illumination, and  $F_u$  the calculated value for the final unquenched fluorescence. For all panels the solid lines are the second rate equations. Data are redrawn from Ruban & Horton (1999).

#### 4. AN ALLOSTERIC MODEL FOR ENERGY-DEPENDENT QUENCHING

This article expands on the principles of enzymology that have been introduced into a previous model for  $q_E$  (Horton *et al.* 1991; Horton & Ruban 1992; Ruban & Horton 1995). It presents a quantitative model for  $q_E$ , derived from kinetic measurements on isolated thylakoids, and shows how this model can explain all of the observations made on the regulation of  $q_E$ . It demonstrates how analysis of isolated proteins can be used to further understand this regulatory mechanism.

The basic features of a regulatory enzyme are uncovered by steady-state kinetic measurements, determining the rate of reaction against substrate concentration, and examining the effects of regulatory effectors. For  $q_E$ , the extent of quenching can be considered the reaction rate. Since there is an obligatory requirement for protonation it is best to consider this as equivalent to a substrate binding because in effect binding a proton gives rise to the formation of the product (the quenched state).

It is relatively simple to determine the quantitative relationship between proton concentration and  $q_E$  in isolated chloroplasts. In figure 1, the results of a titration of  $q_E$  against  $\Delta\text{pH}$  in spinach chloroplasts are shown. The startling feature of the titration curve is the sigmoidicity, which suggests positive cooperativity with respect to proton binding. We have noticed that the extent of sigmoidicity varies, and this is evident also from examination of published data (Krause *et al.* 1988; Noctor *et al.* 1991; Schonknecht *et al.* 1996; Heinze & Dau 1996). The apparent pK can also be calculated from such data, and again it has been shown that the pK varies under different experimental conditions (Krause *et al.* 1988). Shifts to either higher or lower pH have been observed, and for example can be brought about systematically by the  $q_E$  inhibitor antimycin A.

*In vivo*, the xanthophyll cycle carotenoids are the regulators of  $q_E$ . A shift in the titration curve to lower  $\Delta\text{pH}$  upon violaxanthin de-epoxidation was observed in previous work (Rees *et al.* 1989; Noctor *et al.* 1991). In addition to shifting the curve, there is a distinct alteration in kinetics—the titration curve now has reduced sigmoidicity, with the  $\text{H}^+$  binding now approaching 'Michaelis-Menten' kinetics (figure 1). It is very significant that the inhibitory effect of antimycin A depends upon the de-epoxidation state, showing the antagonism between the stimulators and inhibitors of  $q_E$  (Noctor *et al.* 1993).

All of this data can be explained by a simple model for  $q_E$  in which zeaxanthin is an allosteric activator of  $q_E$  (figure 2). Two forms of the PS II antenna are defined, the U form capable only of light harvesting, and the Q form, capable also of energy dissipation. These two states are defined as having different fluorescence lifetimes (Gilmore *et al.* 1995, 1998). Proton binding induces the change in conformation from U to Q. Interaction between LHC subunits gives rise to the positive cooperativity of this process, perhaps through a concerted switch in conformation. Zeaxanthin binding also leads to the change in conformation by preferential binding to the Q state. Conversely, violaxanthin is associated with the U state. The effect of antimycin A is explained either by it binding preferentially to the U state or by it stabilizing this state.

Dibucaine is another reagent to affect  $q_E$ . Although it is not possible to estimate  $\Delta\text{pH}$  accurately in its presence (Gilmore & Yamasaki 1998),  $q_E$  is formed at much lower light intensity (data not shown), which suggests a marked shift in the titration curve. Dibucaine is proposed to promote the transition to the Q state, perhaps by aiding protonation.

#### 5. FEATURES OF ENERGY-DEPENDENT QUENCHING EXPLAINED BY THE ALLOSTERIC MODEL

This simple model readily explains all the features of  $q_E$  encountered in leaves and isolated chloroplasts.

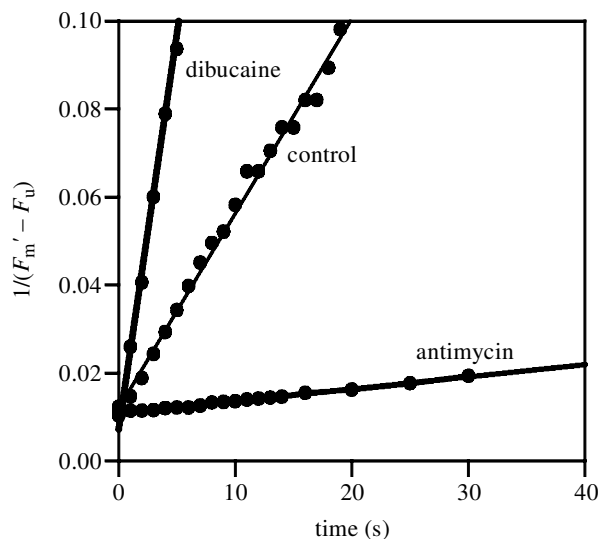


Figure 4. Second-order plots of fluorescence quenching in spinach chloroplasts, showing the effects of dibucaine and antimycin A. The data were obtained as described by Noctor *et al.* 1993. The second-order plot was obtained as described in figure 3.

(a) *Correlation between energy-dependent quenching and de-epoxidation state*

Under low  $\Delta\text{pH}$  conditions,  $q_E$  would be absolutely dependent on the de-epoxidation state. *In vivo*, the impressive correlations between  $q_E$  and de-epoxidation state (e.g. Demmig-Adams *et al.* 1996) can be explained by the low  $\Delta\text{pH}$  that must exist under most conditions—in light saturating for photosynthesis the electron transport rate is necessarily close to its maximum capacity, implying minimal restriction by build-up of  $\Delta\text{pH}$ . Indeed, measurements of the half-time for plastoquinol to  $\text{P}_{700}$  electron transfer indicate that electron transport is not limited by internal pH (Foyer *et al.* 1990). Other observations suggest that the lumen pH does not decrease much below 6.0 (Kramer *et al.* 1999). Hence the functional significance of the data in figure 1 is clear—only in the presence of zeaxanthin can the  $\Delta\text{pH}$  in high light give rise to  $q_E$  because in the dark-adapted, zeaxanthin-free state a lumen pH of *ca.* 4.2 would be required for maximum quenching.

(b) *Kinetics of energy-dependent quenching formation in leaves*

The kinetics of formation of  $q_E$  in leaves may give information on the way in which it is regulated (Ruban & Horton 1999). Because of the dependency of  $\Delta\text{pH}$  upon the rate of ATP consumption by carbon assimilation, it is frequently impossible to disentangle the kinetics of  $q_E$  formation from metabolic events. However, in high light a maximum  $\Delta\text{pH}$  will be rapidly formed and maintained, and the kinetics of  $q_E$  formation will yield information on the dynamic relationship between quenching and protonation. Under such conditions,  $q_E$  forms with biphasic kinetics. There is an initially rapid phase that is limited in magnitude in a dark-adapted leaf. This is followed by a slow phase that brings the quenching to its maximum value. The slow phase is associated with zeaxanthin

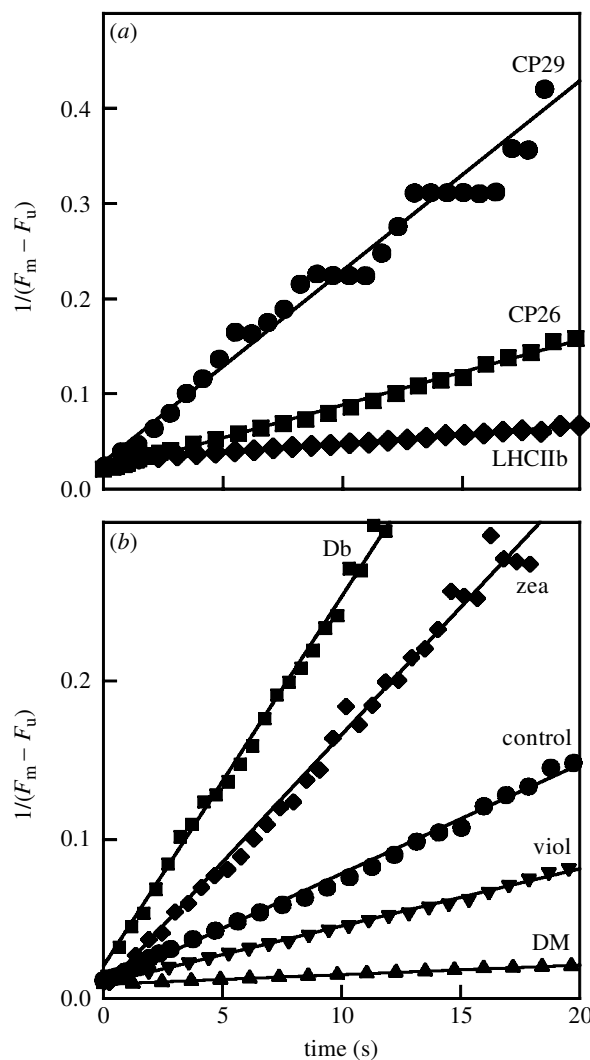


Figure 5. Second-order plots of fluorescence quenching in isolated light-harvesting complexes. (a) Different LHC II samples. (b) The effect of different modulators on CP26: dibucaine (Db), zeaxanthin (zea), viol (violaxanthin) and N-dodecyl  $\beta$ -maltoside (DM). In all cases quenching was initiated at time 0 by dilution of the sample into buffer containing no detergent. Experimental conditions were as described in Ruban *et al.* (1996).

formation, whereas the rapid phase arises because  $\Delta\text{pH}$  alone can bring about some quenching. Illumination of a leaf after a brief dark adaptation results in the maximum level of quenching being reached rapidly. These kinetics are predicted by the allosteric model—at physiological  $\Delta\text{pH}$   $q_E$  formation is limited without the accumulation of zeaxanthin.

(c) *Relaxation kinetics of energy-dependent quenching*

In chloroplasts that have been activated to induce zeaxanthin synthesis,  $q_E$  relaxes more slowly than in chloroplasts containing only violaxanthin (Noctor *et al.* 1991). This can be explained, at least in part, by the higher pK that would require that much more dissipation of  $\Delta\text{pH}$  for  $q_E$  to relax.

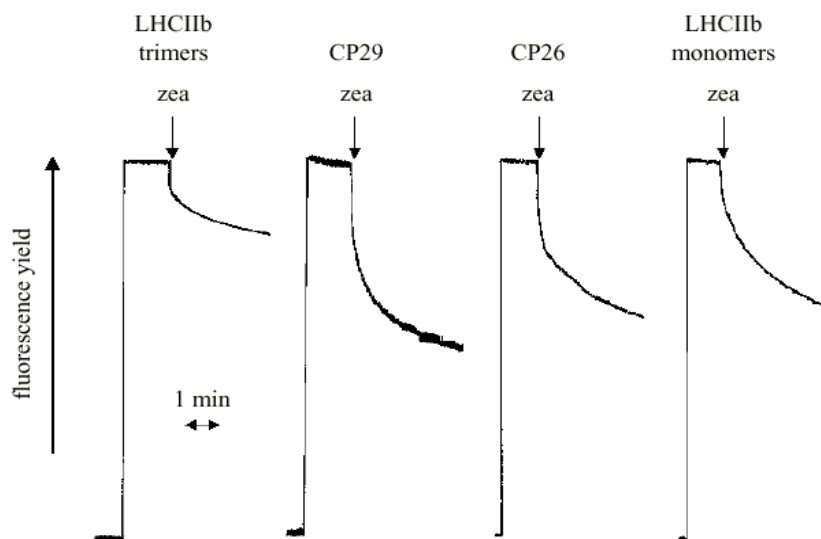


Figure 6. Zeaxanthin-dependent fluorescence quenching in LHC II samples in the presence of 100 mM N-dodecyl  $\beta$ -maltoside, which prevents aggregation of the complexes. The upward increase in fluorescence is the turning on of the measuring beam. LHC IIb monomers were prepared from LHC II trimers by phospholipase treatment. Data are taken from Wentworth *et al.* (2000).

#### (d) Zeaxanthin epoxidase mutants

In *Arabidopsis* mutants lacking zeaxanthin epoxidase, zeaxanthin is present in darkness—upon illumination, there is a rapid formation of  $q_E$ , but the final level is unchanged (Tardy & Havaux 1996; Hurry *et al.* 1997; Niyogi *et al.* 1998). This is predicted by the model since  $q_E$  formation would be only dependent on  $\Delta pH$ .

#### (e) Violaxanthin de-epoxidase mutants

For the same reason, a mutant lacking violaxanthin de-epoxidase would be expected to have a restricted capacity for  $q_E$  under normal  $\Delta pH$  conditions. Examination of the published data on the *Arabidopsis npq1* mutant shows it retains the initial phase of  $q_E$  formation (Niyogi *et al.* 1998), but loses the second phase, again as predicted by the model. In the equivalent mutant of *Chlamydomonas* the capacity for  $q_E$  is hardly affected (Niyogi *et al.* 1997)—this observation can readily be explained by altered dynamics of the model in figure 2.  $\Delta pH$  may be higher in these chloroplasts, or the titration curve in figure 1 shifted to the left so that the dependency upon zeaxanthin formation is reduced.

#### (f) Double mutants lacking lutein

Mutants unable to synthesize lutein have a reduced capacity for  $q_E$  and a slower rate of formation (Pogson *et al.* 1998; Niyogi *et al.* 1997). Whilst such data could be interpreted as indicating a direct quenching role for lutein, we suggest that these mutants are absolutely dependent upon de-epoxidation for  $q_E$  due to altered conformation dynamics of the LHC II proteins, i.e. the titration curve of  $q_E$  against  $\Delta pH$  could be shifted to the right. A double mutant *npq1 lut1* that lacks both zeaxanthin and lutein would hence be  $q_E$  deficient (Niyogi 1999). Interestingly, a *aba1 lut1* double mutant that has a large pool of zeaxanthin and no lutein shows only rapid formation of  $q_E$ , but the total amplitude is greatly reduced (Pogson *et al.* 1998). Whilst not explicitly predicted by the

model, this observation is inconsistent with the simple idea of zeaxanthin and lutein being alternative direct quenchers and points to the importance of the correct structure of the LHC II. For example, the replacement of lutein by zeaxanthin may not allow correct assembly of LHC II, and such alteration may interfere with the U to Q transition.

## 6. KINETIC MODEL FOR ENERGY-DEPENDENT QUENCHING

The approach of steady-state enzyme kinetics therefore allows description of the basic features of the regulation of  $q_E$ . Analysis of pre-steady-state kinetics is the second classical approach to the study of enzyme kinetics. Kinetics of  $q_E$  formation in both leaves and chloroplasts was found to always fit a hyperbolic second-order reaction model (Ruban & Horton 1999). Formation of  $q_E$  is accelerated in the presence of zeaxanthin compared to that found in the presence of violaxanthin even though the  $\Delta pH$  is the same (figure 3). In isolated chloroplasts, dibucaine increases the rate constant for the formation of quenching, whilst antimycin A reduces it (figure 4). The second-order kinetic model is robust and provides the best fit for quenching under all conditions of activation and inhibition. The significance of this will be discussed in § 9.

## 7. TESTING OF THE ALLOSTERIC MODEL USING ISOLATED LIGHT-HARVESTING COMPLEXES

Further insights into the regulation of  $q_E$  have been obtained from observation of the behaviour of LHC II *in vitro* (Ruban *et al.* 1994, 1996; Wentworth *et al.* 2000). Different states of the complexes resemble the putative U and Q forms. Purified complexes at a detergent concentration above the critical micelle concentration (CMC) are highly fluorescent and resemble the U form. Transition to a quenched state can be readily achieved by

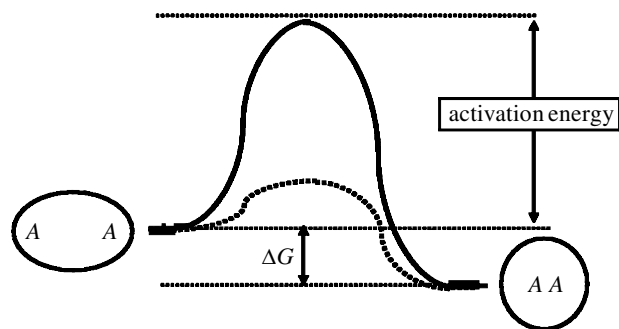


Figure 7. Thermodynamic relationships between the two states of a light-harvesting subunit. In this representation it is proposed that the quenched state is favoured (lower energy). In order to get from the unquenched state, an activation energy barrier needs to be overcome. *In vitro* this can be achieved by an increase in temperature or pressure (solid line) or may be catalysed in the case of  $q_E$  (dotted line).  $\Delta\text{pH}$  and zeaxanthin may be envisaged to increase  $\Delta G$  and/or lowering the activation energy.

dilution below the CMC; the rate of reaching this quenched state can be controlled by the effectors known to control  $q_E$  *in vivo*. Antimycin A is an inhibitor of this spontaneous quenching, and low pH and dibucaine are enhancers (Ruban *et al.* 1994). Zeaxanthin accelerates quenching whilst violaxanthin inhibits it (Ruban *et al.* 1994, 1996). Quenching is inhibited by *N,N'*-dicyclohexylcarbodiimide (DCCD) (Ruban *et al.* 1996, 1998a). Qualitatively similar behaviour is displayed by all LHC II complexes, although quenching is both larger and faster for CP29 and CP26 than for LHC IIb under the same conditions. It is important to note that the kinetics of quenching *in vitro* always fits the same second-order rate equation found for  $q_E$  *in vivo*. The second-order rate constant is modulated by the xanthophylls and by effectors such as antimycin A and dibucaine (figure 5).

Recent work has provided some clues about the nature of the xanthophyll binding to LHC II. For all complexes, the active component of the xanthophyll cycle is rather loosely bound to the complex—it can readily associate and dissociate—and the binding site is proposed to be readily accessible, perhaps on the protein surface (Ruban *et al.* 1999). For the xanthophyll-stimulated quenching *in vitro*, the shape of the carotenoid is of paramount importance in the binding process (Horton *et al.* 1999). An epoxy carotenoid analogue of violaxanthin called auroxanthin was found to have a zeaxanthin-like effect (Ruban *et al.* 1998b). In this carotenoid the epoxide is in the 5–8 position, which holds the end group firmly in the plane of the carbon double bond chain, as in zeaxanthin. In contrast, the end groups of violaxanthin are twisted out of plane. The specificity of the carotenoid effect, and its dependency on structure rather than energy levels, is consistent with the allosteric model for  $q_E$ .

## 8. THE NATURE OF THE STRUCTURAL CHANGE

LHC II is a macromolecular aggregate *in vivo*. Biochemical analysis (Bassi & Dainese 1992), structural studies (Hankamer *et al.* 1997; Boekema *et al.* 1998) and spectroscopic investigation (Garab *et al.* 1988; Kolubayev

*et al.* 1985; Ruban & Horton 1994) have provided evidence for close interaction between the proteins of PS II. A key question to be answered is whether the structural changes associated with the formation of the quenched Q state occur within a protein subunit or whether they involve changes in subunit–subunit interactions. The present authors have attempted to answer this question by investigation of the behaviour of LHC II *in vitro*.

Under the conditions used to induce quenching, large macromolecular protein aggregates are formed (Ruban & Horton 1992). Careful control of detergent concentration enabled conditions to be obtained where the oligomer size was approximately (in the case of LHC IIb) six trimers, as assessed by sucrose gradient centrifugation (Ruban *et al.* 1997). It was demonstrated directly that zeaxanthin promotes oligomer formation, whereas violaxanthin inhibits this process. The conclusion from this data was that these carotenoids act on the periphery of the complexes to control subunit–subunit interaction—such interaction was presumed to be obligatory for quenching. Q was suggested to be an aggregated state of LHC II.

Subsequently, however, conditions were found where quenching could be induced by zeaxanthin in the absence of protein aggregation (figure 6; Wentworth *et al.* 2000). This quenching process was again second order, and gave rise to exactly the same changes in absorption spectrum as for ‘aggregation’-dependent quenching. It was found that zeaxanthin binding under these conditions also increased the rate of quenching if the complex was subsequently exposed to aggregation conditions. On the basis of these observations we suggest that the quenching event occurs within a single protein subunit. Furthermore, we suggest that the change in conformation associated with the quenched Q state has an increased tendency for aggregation. Aggregation would pull the equilibrium in favour of the Q state.

## 9. THE SIGNIFICANCE OF SECOND-ORDER KINETICS

The hyperbolic kinetics of quenching indicates a special type of second-order reaction of the type  $A + A \rightarrow 2A$ , where A is a highly fluorescent species, found in the U form, and 2A occurs in the Q form. Since quenching can occur within a protein monomer, then A must be a domain within a protein. Hence the simplest explanation of quenching is that it results from a conformational change that brings together two A domains to form the quencher 2A. For example, A may be a chlorophyll molecule bound to a chlorophyll protein complex, and 2A a chlorophyll dimer.

## 10. TWO STATES OF THE PS II ANTENNA

The transition between U and Q must obey the basic principles of reaction thermodynamics (figure 7). Under conditions favouring quenching there must be a significant difference between the free energies of the U and Q states. This difference is enhanced, for example, by protonation and by zeaxanthin binding. The transition would involve an activation energy barrier that must be overcome. For isolated proteins, the frequency of crossing this barrier should be raised by increase in temperature and pressure. Hence, it is particularly significant that

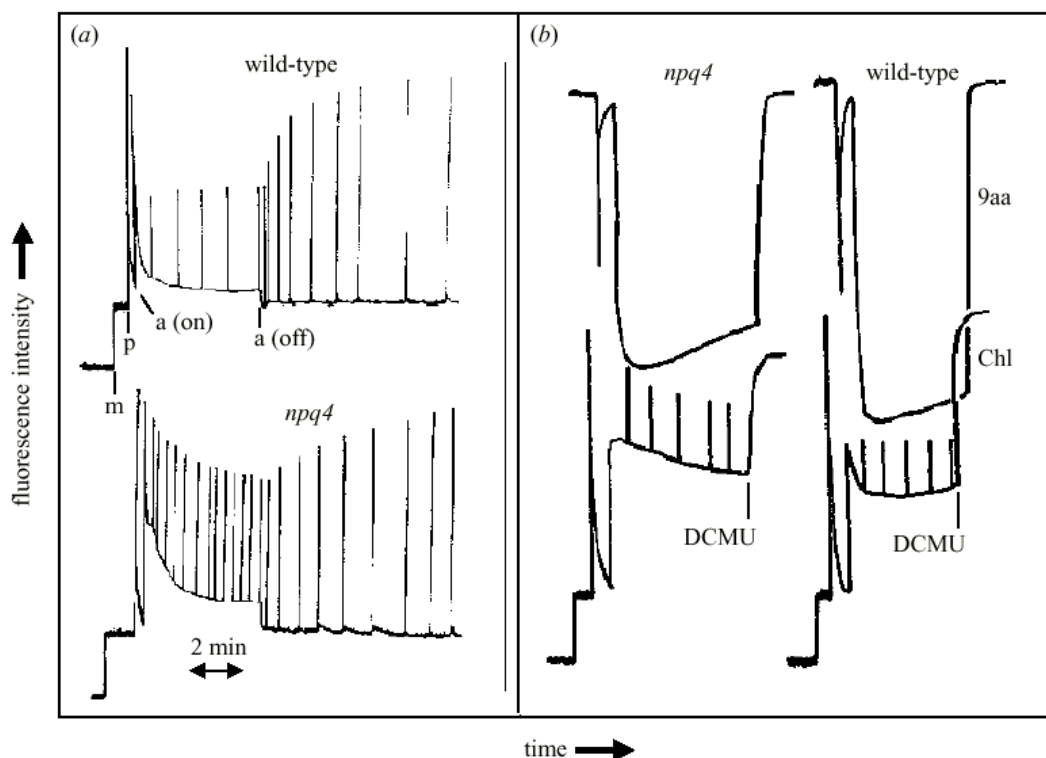


Figure 8. Fluorescence quenching in (a) leaves and (b) isolated chloroplasts of wild-type and *npq4* mutant of *Arabidopsis thaliana*. Plants were grown at  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  for six weeks at  $20^\circ\text{C}/15^\circ\text{C}$  day/night temperature with an 8 h photoperiod. Chloroplasts were isolated according to Kunst (1998). Measurements on leaves were made in air at  $22^\circ\text{C}$  at an irradiance (defined by an RG630 filter) of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  using attached leaves; the traces shown are for a re-illumination after an initial 10 min period of illumination followed by a 10 min dark adaptation (see rationale in Ruban & Horton (1999)). Measurements on chloroplasts were carried out essentially as described by Noctor *et al.* (1991) with an actinic irradiance of  $600 \text{ mol m}^{-2} \text{s}^{-1}$  defined by an RG630 filter. 9aa, fluorescence from 9-aminoacridine; Chl, chlorophyll fluorescence; m, measuring beam on; p, light saturation pulse; a, actinic light on and off. DCMU ( $10 \mu\text{M}$ ) was added to inhibit electron transport and cause the collapse of  $\Delta\text{pH}$ .

quenching in LHC IIb has been shown to be induced by an increase in pressure (Connelly *et al.* 1998). Quenching in isolated LHC IIb can also be induced by strong light (Jennings *et al.* 1991) and this may arise from a localized increase in temperature within the complex (G. Garab, personal communication). Enzymes function by lowering the activation energy of a reaction, and it is therefore pertinent to consider whether, *in vivo*, the transition is catalysed: Are there specific PS II proteins that interact with the LHC II components so as to promote the change in conformation? Below it is suggested that PsbS may be such a catalyst.

#### 11. WHICH PROTEINS ARE INVOLVED IN NON-PHOTOCHEMICAL QUENCHING?

*In vitro* studies indicate that all Lhcb proteins have the capacity to be converted into the quenched state. The key question is whether this potential is expressed *in vivo*. Examination of mutants deficient in LHC IIb indicate that these proteins are not required for quenching, although the efficiency of quenching is reduced in their absence (for a review, see Horton *et al.* 1999). Elimination of CP29 and CP26 by expression of antisense genes has only small effects on  $q_E$  (J. Andersson, S. Jansson, R. G. Walters, A. V. Ruban and P. Horton, unpublished data), a surprising observation given the inhibition of  $q_E$  by DCCD, which binds to these two proteins (Walters *et al.* 1994). The

simplest conclusion to be made from these observations is that none of the LHC II components provides the site of quenching and instead another protein is responsible for the U to Q transition. However, until more is learned about the macromolecular organization of the LHC II system, it is perhaps premature to come to such a conclusion. We have suggested that efficient formation and relaxation of  $q_E$ , and its allosteric behaviour, requires the macromolecular organization of PS II in the grana (Horton *et al.* 1994; Horton 1999), that is in turn dependent on the presence of several types of Lhcb proteins.

#### 12. HOW IS THE *npq4* PHENOTYPE EXPLAINED?

The *npq4* mutant presents a unique opportunity to examine the basic features of the allosteric model. PsbS has been reported to form CP22 (Funk *et al.* 1995), a pigment-binding protein with strong structural homology to LHC II, except that it possesses four transmembrane helices (Kim *et al.* 1992). It has not been shown whether it binds zeaxanthin, it does not bind DCCD, a  $q_E$  inhibitor (A. V. Ruban, unpublished data) and appears not to contain the DCCD-binding amino-acid residues found in CP29 and CP26. The chlorophyll-binding properties of PsbS are also unclear with the published absorption spectra of this protein differing widely.

In the absence of PsbS the rapidly relaxing  $q_E$  component of NPQ is absent (Li *et al.* 2000; figure 8). However,



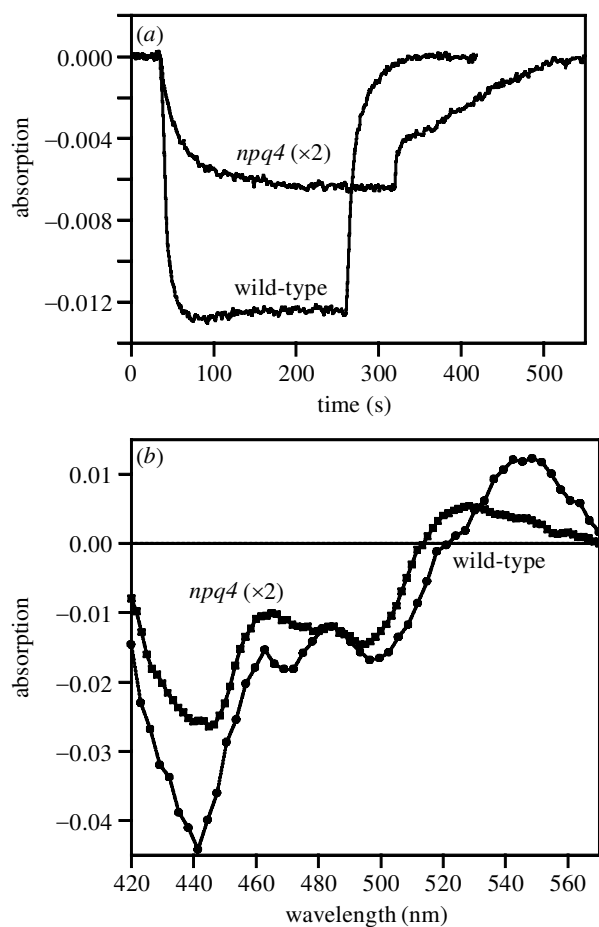


Figure 9. Light-induced absorbance changes for leaves of wild-type and *npq4* mutants of *Arabidopsis thaliana*. For conditions, see figure 8. Absorption measurements were made on leaf pieces as described by Ruban *et al.* (1993). (a)  $\Delta A$  at 505 nm minus 535 nm, downward deflection for light on, upward deflection for light off. The 10 min pre-illumination was used to saturate the synthesis of zeaxanthin, which therefore does not contribute to the absorbance change in the second illumination. (b) Light minus dark difference spectra recorded for a series of experiments at difference measuring wavelengths.

some NPQ remains, and careful examination shows it to be  $q_E$  in type. This NPQ is slow to form and slow to relax, but is prevented by infiltration of leaves with the uncoupling agent nigericin (not shown). Examination of isolated chloroplasts confirms the presence of  $\Delta pH$ -dependent NPQ in the mutant, although its amplitude is substantially less than for the wild-type. Thus addition of *N*-(3,4-dichlorophenyl)-*N'*-dimethylurea caused rapid relaxation of the  $\Delta pH$  and of NPQ in both the wild-type and *npq4*. We have also found that NPQ is enhanced by dibucaine and inhibited by antimycin A in both wild-type and *npq4* chloroplasts, suggesting that the quenches both arise from a similar process.

NPQ in both plant types was also associated with absorption changes in the 400–550 nm region (figure 9). The absorption changes in this region are complex, and originate at least in part from alteration in the macrostructure of the thylakoid membrane. A number of studies have related the absorbance changes to  $q_E$  (Bilger

& Björkman 1994; Ruban *et al.* 1993), and this represents the principal line of evidence that conformational changes are involved. The distinct positive band at 535 nm seen in wild-type plants is reduced and blue-shifted, whereas the negative features in the 400–500 nm region are rather similar but of smaller amplitude. It is very significant that the kinetics of NPQ formation and relaxation correlated with the kinetics of changes in absorbance in both the mutant and wild-type. Thus in *npq4*, induction of quenching is associated with absorbance changes that are qualitatively similar to those observed in the wild-type.

At present it is not possible to distinguish between two alternative explanations of the *npq4* phenotype.

- (i) PsbS is the unique site of rapidly forming  $q_E$ , but other sites may occur in other PS II proteins. These sites are relatively weak quenchers and therefore probably of little physiological importance. To prove this idea, it would need to be demonstrated that PsbS binds pigments that can accept and quench excitation energy from the light-harvesting system. One attractive rationale is that PsbS is a 'primitive' protein evolved as a photoprotective quencher and it was the ancestor of the LHC proteins that has lost the full capacity for quenching (S. Jansson, personal communication). Hence the *in vitro* behaviour of LHC II represents a vestigial feature of the PsbS function. It is interesting that the LHC IIb trimer, the most advanced light harvester, is the most resistant to quenching *in vitro* (Ruban *et al.* 1996; Wentworth *et al.* 2000).
- (ii) Alternatively, PsbS may be necessary to enable  $q_E$  to take place efficiently. Perhaps it is the catalyst of the U to Q transition described in § 10. In the absence of PsbS some  $q_E$  would take place, but the rate of formation would be slow. The final Q form may also be a less efficient quencher, because of the 'misorganization' of the condensed state of the thylakoid membrane (this would be reflected in the altered spectrum for the light induced  $\Delta A$  in figure 9). The present authors have obtained some preliminary evidence that is consistent with PsbS having an influence on the macrostructure of the thylakoid membrane (P. Horton, A. V. Ruban and M. Wentworth, unpublished data). The membrane of the *npq4* mutant is more resistant to detergent solubilization than the wild-type membranes. With the development of new methods to determine the macromolecular structure of the PS II complexes *in vivo*, it may be possible to determine directly if the thylakoid membrane organization is altered in the *npq4* mutant.

### 13. CONCLUSION

The regulation of light harvesting can be described in terms of the concepts and rationale of the study of enzyme-catalysed reactions. This approach not only explains the role of the xanthophyll cycle but also provides a framework for understanding the transitions between two fundamentally contrasting states of the light-harvesting system. The results obtained with a variety of

plant mutants can also be readily accommodated into this model without the need to invoke additional complexity associated with additional quenching mechanisms. The isolation of a mutant with drastically altered kinetics and amplitude of quenching provides an important challenge to this explanation of  $q_E$ . The fact that the absorbance changes associated with membrane energization are perturbed in this mutant points to a special role for the macro-organization of PS II in defining these two states. Thus regulation of light harvesting of the NPQ-type is associated with (i) the presence of proteins encoded by the LHC super gene family (Jansson 1999); (ii) the operation of the xanthophyll cycle; (iii) the formation of a pH difference across the thylakoid membrane; and (iv) the organization of the chlorophyll proteins into the macrostructure associated with grana (Horton 1999). Although we do not yet know the degree of interdependency of these phenomena, it is possible that they are all necessary for NPQ, and that these are a suite of adaptations of oxygenic photosynthesis that allows efficient light harvesting in limiting light and photoprotection in high light. The observation that NPQ can apparently be eliminated and, after a suitable acclimation process, replaced by other (perhaps more evolutionarily primitive) photoprotective processes associated with electron transport or O<sub>2</sub> radical scavenging, without deleterious effects on the performance of plants in excess light, is not inconsistent with such a view. Photoprotection is so important to plant survival that the existence of several levels of defence would be predicted.

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## REFERENCES

- Bassi, R. & Dainese, P. 1992 A supramolecular light-harvesting complex from chloroplast photosystem II membranes. *Eur. J. Biochem.* **204**, 317–326.
- Bilger, W. & Björkman, O. 1994 Relationships among violaxanthin de-epoxidation, thylakoid membrane conformation, and nonphotochemical chlorophyll fluorescence quenching in leaves of cotton (*Gossypium hirsutum* L.). *Planta* **193**, 238–246.
- Boekema, E. J., Van Roon, H. & Dekker, J. P. 1998 Specific association of photosystem II and light-harvesting complex II in partially solubilized photosystem II membranes. *FEBS Lett.* **424**, 95–99.
- Connelly, J. P., Ruban, A. V. & Horton, P. 1998 Pressure induced fluorescence quenching in plant light harvesting complex II. In *High pressure food science, bioscience and chemistry* (ed. N. S. Isaacs), pp. 417–422. Cambridge, UK: The Royal Society of Chemistry.
- Demmig-Adams, B. 1990 Carotenoids and photoprotection in plants—a role for the xanthophyll zeaxanthin. *Biochim. Biophys. Acta* **1020**, 1–24.
- Demmig-Adams, B., Gilmore, A. & Adams III, W. W. 1996 In vivo functions of carotenoids in higher plants. *FASEB J.* **10**, 403–412.
- Foyer, C., Furbank, R., Harbinson, J. & Horton, P. 1990 The mechanisms contributing to photosynthetic control of electron-transport by carbon assimilation in leaves. *Photosynth. Res.* **25**, 83–100.
- Frank, H. A., Cua, A., Chynwat, V., Young, A., Gosztola, D. & Wasielewski, M. R. 1994 Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. *Photosynth. Res.* **41**, 389–395.
- Funk, C., Schroder, W. P., Napiwotzki, A., Tjus, S. E., Renger, G. & Andersson, B. 1995 The PS II-S protein of higher-plants—a new-type of pigment-binding protein. *Biochemistry* **34**, 11 133–11 141.
- Garab, G., Faludi Daniel, A., Sutherland, J. C. & Hind, G. 1988 Macroorganization of chlorophyll a/b light-harvesting complex in thylakoids and aggregates—information from circular differential scattering. *Biochemistry* **27**, 2425–2430.
- Gilmore, A. M. & Yamamoto, H. Y. 1992 Linear models relating xanthophylls and lumen acidity to non photochemical fluorescence quenching: evidence that antheraxanthin explains zeaxanthin independent quenching. *Photosynth. Res.* **35**, 67–78.
- Gilmore, A. M. & Yamasaki, H. 1998 9-aminoacridine and dibucaine exhibit competitive interactions and complicated inhibitory effects that interfere with measurements of delta pH and xanthophyll cycle-dependent photosystem II energy dissipation. *Photosynth. Res.* **57**, 159–174.
- Gilmore, A. M., Hazlett, T. L. & Govindjee 1995 Xanthophyll cycle-dependent quenching of photosystem-ii chlorophyll-a fluorescence—formation of a quenching complex with a short fluorescence lifetime. *Proc. Natl Acad. Sci. USA* **92**, 2273–2277.
- Gilmore, A. M., Shinkarev, V. P., Hazlett, T. L. & Govindjee 1998 Quantitative analysis of the effects of intrathylakoid pH and xanthophyll cycle pigments on chlorophyll a fluorescence lifetime distributions and intensity in thylakoids. *Biochemistry* **37**, 13 582–13 593.
- Hankamer, B., Barber, J. & Boekema, E. J. 1997 Structure and membrane organisation of photosystem II in green plants. *A. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 641–671.
- Heinze, I. & Dau, H. 1996 The pH-dependence of the photosystem II fluorescence: co-operative transition to a quenching state. *Berichte Der Bunsen-Gesellschaft-Physical Chemistry Chemical Physics* **100**, 2008–2013.
- Horton, P. 1985 Interactions between electron transport and carbon metabolism. In *Topics in photosynthesis. VI. Photosynthetic mechanisms and the environment* (ed. J. Barber & N. R. Baker), pp. 135–187. Amsterdam, The Netherlands: Elsevier.
- Horton, P. 1999 Are grana necessary for regulation of light harvesting? *Aust. J. Plant Physiol.* **26**, 659–669.
- Horton, P. 2000 Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. *J. Exp. Bot.* **51**, 475–485.
- Horton, P. & Ruban, A. V. 1992 Regulation of photosystem II. *Photosynth. Res.* **34**, 375–385.
- Horton, P., Ruban, A. V., Rees, D., Pascal, A. A., Noctor, G. & Young, A. J. 1991 Control of the light-harvesting function of chloroplast membranes by aggregation of the LHC II chlorophyll protein complex. *FEBS Lett.* **292**, 1–4.
- Horton, P., Ruban, A. V. & Walters, R. G. 1994 Regulation of light-harvesting in green plants—indication by non-photochemical quenching of chlorophyll fluorescence. *Plant Physiol.* **106**, 415–420.
- Horton, P., Ruban, A. V. & Walters, R. G. 1996 Regulation of light harvesting in green plants. *A. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 655–684.
- Horton, P., Ruban, A. V. & Young, A. J. 1999 Regulation of the structure and function of the light harvesting complexes of photosystem II by the xanthophyll cycle. In *The photochemistry of carotenoids* (ed. H. A. Frank, A. J. Young & R. J. Cogdell), pp. 271–291. Dordrecht, The Netherlands: Kluwer.
- Hurry, V., Anderson, J. M., Chow, W. S. & Osmond, C. B. 1997 Accumulation of zeaxanthin in abscisic acid-deficient mutants of *Arabidopsis* does not affect chlorophyll fluorescence

- quenching or sensitivity to photoinhibition *in vivo*. *Plant Physiol.* **113**, 639–648.
- Jansson, S. 1999 A guide to the Lhc genes and their relatives in *Arabidopsis*. *Trends Plant Sci.* **4**, 236–240.
- Jennings, R. C., Zucchelli, G. & Garlaschi, F. M. 1991 Light-induced fluorescence quenching in the light-harvesting chlorophyll a/b protein complex. *Photosynth. Res.* **27**, 57–64.
- Kim, S., Sandusky, P., Bowlby, N. R., Absersold, R. & Green, B. R. 1992 Characterisation of a spinach psbS cDNA encoding the 22 kDa protein of photosystem II. *FEBS Lett.* **314**, 67–71.
- Kolubayev, T., Geacintov, N. E., Paillotin, G. & Breton, J. 1985 Domain sizes in chloroplasts and chlorophyll-protein complexes probed by fluorescence yield quenching induced by singlet-triplet exciton annihilation. *Biochim. Biophys. Acta* **808**, 66–76.
- Kramer, D. M., Sacksteder, C. A. & Cruz, J. A. 1999 How acidic is the lumen? *Photosynth. Res.* **60**, 151–163.
- Krause, G. H., Laasch, H. & Weis, E. 1988 Regulation of thermal dissipation of absorbed light energy in chloroplasts indicated by energy-dependent fluorescence quenching. *Plant Physiol. Biochem.* **26**, 445–452.
- Kunst, L. 1998 Preparation of physiologically active chloroplasts from *Arabidopsis*. In *Methods in molecular biology*. 82. *Arabidopsis protocols* (ed. J. Martinez-Zapater & J. Salinas), pp.43–53. Totoway, NJ: Humana Press, Inc.
- Li, X. P., Björkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S. & Niyogi, K. K. 2000 A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* **403**, 391–395.
- Niyogi, K. K. 1999 Photoprotection revisited: genetic and molecular approaches. *A. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 333–359.
- Niyogi, K. K., Björkman, O. & Grossman, A. R. 1997 The roles of specific xanthophylls in photoprotection. *Proc. Natl Acad. Sci. USA* **94**, 14162–14167.
- Niyogi, K. K., Grossman, A. R. & Björkman, O. 1998 *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *Plant Cell* **10**, 1121–1134.
- Noctor, G., Rees, D., Young, A. J. & Horton, P. 1991 The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence and the transthylakoid pH-gradient in isolated chloroplasts. *Biochim. Biophys. Acta* **1057**, 320–330.
- Noctor, G., Ruban, A. V. & Horton, P. 1993 Modulation of delta-pH-dependent nonphotochemical quenching of chlorophyll fluorescence in spinach-chloroplasts. *Biochim. Biophys. Acta* **1183**, 339–344.
- Owens, T. G., Shreve, A. P. & Albrecht, A. C. 1992 Dynamics and mechanism of singlet energy transfer between carotenoids and chlorophylls: light harvesting and nonphotochemical fluorescence quenching. In *Research in photosynthesis*, vol. 4 (ed. N. Murata), pp.179–186. Dordrecht, The Netherlands: Kluwer.
- Pesaresi, P., Sandona, D., Giuffra, E. & Bassi, R. 1997 A single point mutation (E166Q) prevents dicyclohexylcarbodiimide binding to the photosystem II subunit CP29. *FEBS Lett.* **402**, 151–156.
- Pogson, B. J., Niyogi, K. K., Björkman, O. & DellaPenna, D. 1998 Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. *Proc. Natl Acad. Sci. USA* **95**, 13 324–13 329.
- Polívka, T., Herek, J. L., Zigmantas, D., Åkerlund, H. E. & Sundström, V. 1999 Direct observation of the (forbidden) S-1 state in carotenoids. *Proc. Natl Acad. Sci. USA* **96**, 4914–4917.
- Rees, D., Young, A., Noctor, G., Britton, G. & Horton, P. 1989 Enhancement of the delta-pH-dependent dissipation of excitation-energy in spinach-chloroplasts by light-activation—correlation with the synthesis of zeaxanthin. *FEBS Lett.* **256**, 85–90.
- Ruban, A. V. & Horton, P. 1992 Mechanism of delta-pH-dependent dissipation of absorbed excitation-energy by photosynthetic membranes. 1. Spectroscopic analysis of isolated light-harvesting complexes. *Biochim. Biophys. Acta* **1102**, 30–38.
- Ruban, A. V. & Horton, P. 1994 Spectroscopy of nonphotochemical and photochemical quenching of chlorophyll fluorescence in leaves—evidence for a role of the light-harvesting complex of photosystem II in the regulation of energy dissipation. *Photosynth. Res.* **40**, 181–190.
- Ruban, A. V. & Horton, P. 1995 Regulation of nonphotochemical quenching of chlorophyll fluorescence in plants. *Aust. J. Plant Physiol.* **22**, 221–230.
- Ruban, A. V. & Horton, P. 1999 The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach. *Plant Physiol.* **119**, 531–542.
- Ruban, A. V., Young, A. J. & Horton, P. 1993 Induction of non-photochemical energy-dissipation and absorbency changes in leaves—evidence for changes in the state of the light-harvesting system of photosystem II *in-vivo*. *Plant Physiol.* **102**, 741–750.
- Ruban, A. V., Young, A. J. & Horton, P. 1994 Modulation of chlorophyll fluorescence quenching in isolated light-harvesting complex of photosystem-II. *Biochim. Biophys. Acta* **1186**, 123–127.
- Ruban, A. V., Young, A. J. & Horton, P. 1996 Dynamic properties of the minor chlorophyll a/b binding proteins of photosystem II, an *in vitro* model for photoprotective energy dissipation in the photosynthetic membrane of green plants. *Biochemistry* **35**, 674–678.
- Ruban, A. V., Phillip, D., Young, A. J. & Horton, P. 1997 Carotenoid-dependent oligomerization of the major chlorophyll a/b light harvesting complex of photosystem II of plants. *Biochemistry* **36**, 7855–7859.
- Ruban, A. V., Pesaresi, P., Wacker, U., Irrgang, K. J., Bassi, R. & Horton, P. 1998a The relationship between the binding of dicyclohexylcarbodiimide and quenching of chlorophyll fluorescence in the light-harvesting proteins of photosystem II. *Biochemistry* **37**, 11 586–11 591.
- Ruban, A. V., Phillip, D., Young, A. J. & Horton, P. 1998b Excited-state energy level does not determine the differential effect of violaxanthin and zeaxanthin on chlorophyll fluorescence quenching in the isolated light-harvesting complex of photosystem II. *Photochem. Photobiol.* **68**, 829–834.
- Ruban, A. V., Lee, P. J., Wentworth, M., Young, A. J. & Horton, P. 1999 Determination of the stoichiometry and strength of binding of xanthophylls to the photosystem II light harvesting complexes. *J. Biol. Chem.* **274**, 10 458–10 465.
- Schonknecht, G., Neimanis, S., Katona, E., Gerst, U. & Heber, U. 1995 Relationship between photosynthetic electron transport and pH gradient across the thylakoid membrane in intact leaves. *Proc. Natl Acad. Sci. USA* **92**, 12 185–12 189.
- Tardy, F. & Havaux, M. 1996 Photosynthesis, chlorophyll fluorescence, light-harvesting system and photoinhibition resistance of a zeaxanthin-accumulating mutant of *Arabidopsis thaliana*. *J. Photochem. Photobiol. B* **34**, 87–94.
- Walters, R. G., Ruban, A. V. & Horton, P. 1994 Higher-plant light-harvesting complexes LHC IIa and LHC IIc are bound by dicyclohexylcarbodiimide during inhibition of energy-dissipation. *Eur. J. Biochem.* **226**, 1063–1069.
- Walters, R. G., Ruban, A. V. & Horton, P. 1996 Identification of proton-active residues in a higher plant light-harvesting complex. *Proc. Natl Acad. Sci. USA* **93**, 14 204–14 209.
- Wentworth, M., Ruban, A. V. & Horton, P. 2000 Chlorophyll fluorescence quenching in isolated light harvesting complexes induced by zeaxanthin. *FEBS Lett.* **471**, 71–74.