Biochimica et Biophysica Acta 932 (1988) 107–115
Elsevier

BBA 42695

Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching

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(Received 30 June 1987)

Key words: Photosynthesis; Chlorophyll fluorescence; Fluorescence quenching; (Barley protoplast)

Using DCMU addition and light-saturation pulses, chlorophyll fluorescence quenching by isolated barley protoplasts has been examined upon illumination with different light intensities. Total quenching was constant at all intensities, photochemical quenching predominating in low light and non-photochemical quenching becoming increasingly important at higher intensity. The components of non-photochemical quenching were resolved by examination of kinetics of relaxation of quenching upon DCMU addition or darkening. It was found that: (a) energy-dependent quenching saturated with characteristics similar to photosynthesis; (b) a slowly relaxing, NaF-sensitive component attributed to protein phosphorylation saturated in low light and decreased at high light; (c) an irreversible component ascribed to photoinhibition was a major quencher as light levels were increased above saturation; (d) the decreased photochemical quenching at high light was not strictly correlated with alteration in rate of O_2 evolution. Analysis of these data showed that the quantum yield of Photosystem II declined as the light intensity was increased from zero to that saturating for photosynthesis and was associated with the presence of energy-dependent quenching. The regulation of excitation dissipation by the thylakoid membrane is discussed.

Introduction

Upon illumination of intact chloroplasts, protoplasts and leaves, there is an induction period before the maximum steady-state rate of photosynthesis is attained (for a review see Ref. 1). During this period the yield of chlorophyll fluorescence is quenched from a level F_p seen within seconds of illumination to a steady-state level F_t (for reviews, see Refs. 2 and 3). This quenching of fluorescence has been ascribed to two kinds of processes [4,5]. Firstly, the acceleration of photosynthesis results in increased utilization of excitation photochemically, a process defined as qQ quenching. Secondly, excitation can be dissipated by a variety of mechanisms which are non-photochemical in nature. The major one of these is caused by the energisation of the thylakoid membrane by light-driven H^+ uptake and is called qE. In addition, there is a decrease in the proportion of excitation transferred to PS II, a process determined by protein phosphorylation, and termed qT. Finally, photoinhibition of PS II is also associ-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS II, Photosystem II; F_m, fluorescence level when all PS II centres are closed; F_o, fluorescence level when all centres are open; qQ, photochemical quenching; qNP, non-photochemical quenching; qE, energy-dependent quenching; qT, state-transition-dependent quenching; qI, photoinhibition-dependent quenching; qR, 'remaining quenching'; LHC II, light-harvesting chlorophyll-protein complex of Photosystem II; Chl, chlorophyll.

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ated with a decline in fluorescence yield, qI.

A study of fluorescence quenching in isolated protoplasts has, by use of the techniques of DCMU or light addition, quantified the contributions of qQ and qE to the fluorescence induction curve [6–8] and these have been related to metabolic changes in the chloroplast stroma and cytoplasm [9]. However, a proportion of fluorescence quenching was observed that was neither qQ nor qE and was termed qR [7,8]. In this paper experiments are described which attempt to resolve qR into changes due to protein phosphorylation (qT) and photoinhibition (qI). Also of interest has been to investigate further the constancy of the sum of all the quenching processes; in earlier work the total amount of quenching was constant [7], suggesting that in some way the non-photochemical processes were involved in controlling excitation dissipation [10]. This suggestion has been strengthened by recent reports that qE lowers the quantum yield of PS II photochemistry [11], inhibits photoinhibitory quenching [12] and suppresses protein phosphorylation [10,13]. A general hypothesis was put forward to suggest that each quenching mechanism is a physiological response to increased light intensity, each one with a different response time and response range [10]. In this paper, further evidence to support this notion is presented.

Materials and Methods

Protoplasts were isolated from barley (*Hordeum vulgare* L. cv Marko) exactly as described previously [6]. Measurements of the rate of O_2 evolution and the intensity of chlorophyll fluorescence were performed simultaneously at 20 °C as previously described [7,14], except that an aluminium chamber (Hansatech DW2) was used. Chlorophyll fluorescence was excited using an array of yellow, modulated light-emitting diodes and detected with a photodiode through an RG715 filter using the Hansatech Modulated Fluorometer [15]. The intensity of the modulated measuring beam was adjusted to an intensity below which no further decrease resulted in a larger F_v/F_m ratio. Application of a backround far-red light was not able to lower the fluorescence below that observed with the measuring beam on. The fluorescence level recorded was therefore very close to F_0; close examination indicated that this level was approx. 5–10% above the true F_0 level. An approximation of the F_0 during steady-state illumination was made by recording the lowest fluorescence level reached immediately upon turning the light off [16]. Red actinic light was provided by a heat-filtered 250 W tungsten-halogen lamp and defined by a Schott RG610 and an Ealing 660 short-pass filters giving a maximum intensity of 1400 μmol quanta $\cdot s^{-1} \cdot m^{-2}$. An identical second light source could provide, by means of a Uniblitz 15 mm shutter, saturating pulses of light (1 s duration, intensity 1800 μmol quanta $\cdot s^{-1} \cdot m^{-2}$) given at frequencies of 0.125 Hz or lower (this frequency was adjusted as described in the text according to the actinic light intensity employed). The O_2 electrode signal was amplified and differentiated electronically before being fed to a Rikadenki four-channel chart recorder.

Results

Fig. 1 shows the fluorescence induction curves of protoplasts illuminated with high light (A) and low light (B). In both cases actinic light raised the fluorescence to a peak and thereafter quenching occurred, with the complex kinetics previously described at length [6]. In both A and B the total extent of quenching at steady state is the same. The use of light pulses resolves non-photochemical quenching and showed clearly that in low light qQ predominates, whereas in high light quenching was mainly non-photochemical. Addition of DCMU allowed further resolution of the quenching curve [7]; the slowly relaxing phase of relaxation defines qE and the difference between the initial F_m and the level reached at the end of this relaxation defines the 'remaining quenching', qR. In high light, qR was quite significant (25% of variable fluorescence and nearly 50% of non-photochemical quenching).

If, after DCMU addition, the actinic light was turned off but pulsing continued, a further phase of relaxation of quenching was detected with a $t_{1/2}$ of approx. 5 min. In low light, relaxation occurred back to the original F_m (Fig. 1B), whereas in high light, there was a significant proportion of 'irreversible' quenching (Fig. 1A). The kinetics of relaxation of the slowly reversible phase resemble

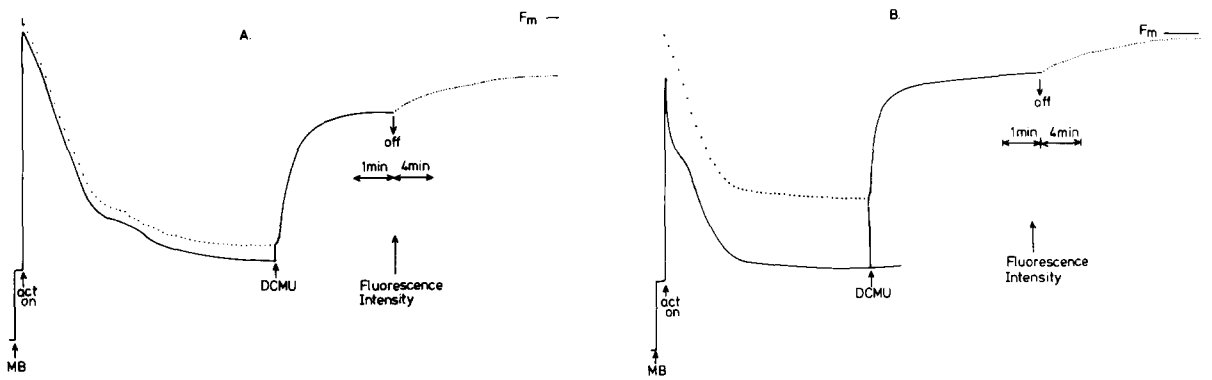

Fig. 1. Chlorophyll fluorescence yield in barley protoplasts illuminated with (A) 1400 and (B) 70 μmol quanta $\cdot s^{-1} \cdot m^{-2}$. At 7 s intervals saturating pulses ($\cdots\cdots$) were given and at steady-state 10 μM DCMU added. After 4 min, the constant light was turned off and pulsing continued for a further 15 min. Note the change in the time scale at this point.

those expected for dephosphorylation of LHC II [17]. Furthermore, addition of NaF completely inhibited this phase of relaxation (Fig. 2); NaF is a potent inhibitor of the thylakoid protein phosphatase [18]. A high concentration of NaF was needed, probably due to the impermeability of the protoplast plasmalemma. It should be noted that there was no overall alteration in fluorescence yield in the presence of NaF as would occur, for example, if there were a non-specific effect on thylakoid organisation due to Mg^{2+} chelation. Previous work has demonstrated the existence of light-induced incorporation of ^{32}P into LHC II

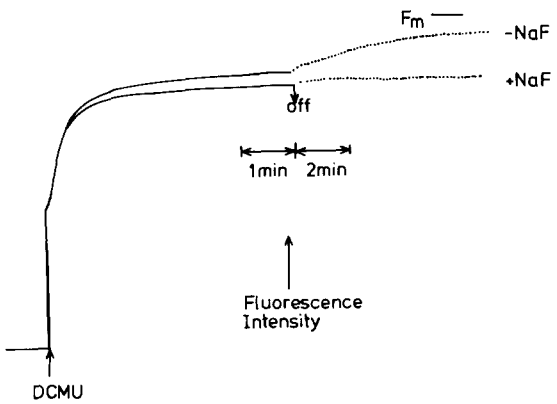

Fig. 2. Effect of NaF on relaxation of fluorescence quenching. The experiment was carried out as in Fig. 1, except that at steady state, and just prior to DCMU addition, 50 mM NaF was added to one of the samples. The light intensity was 70 μmol quanta $\cdot s^{-1} \cdot m^{-2}$.

and changes in the emission spectrum at $-196\,^{\circ}C$ in barley protoplasts [19]. An alternative explanation would be that the relaxation represents recovery from photoinhibition. However, recovery from photoinhibition in barley protoplasts is much slower with $t_{1/2}$ of about 30 min and is completely blocked by chloramphenicol [20]. Chloramphenicol had no effect on the relaxation of quenching shown in Fig. 1 (data not shown).

In Fig. 3, the extents of quenching by different processes as a function of light intensity is presented. The total amount of steady-state quenching of chlorophyll fluorescence was found to be remarkably constant over a 300-fold change in light intensity. Clearly, however, excitation is dissipated through different routes at different light intensity. Dissipation by photosynthesis (Q) became a smaller proportion as the light intensity increased, whereas non-photochemical quenching (qNP) rose complementarily. Irreversible quenching (qI) increased as the light intensity increased, whereas energy-dependent quenching (qE) first rose and then fell. The NaF-sensitive, slowly reversing component (qT) seemed to remain approximately constant over a wide range of light intensities, although close examination indicates that it rose to a maximum value (43% of non-photochemical quenching) in very low light, decreased to a plateau (16% of non-photochemical quenching) between 100 and 1500 μmol quanta $\cdot s^{-1} \cdot m^{-2}$ and fell to 8% of non-photochemical quenching in above-saturating light.

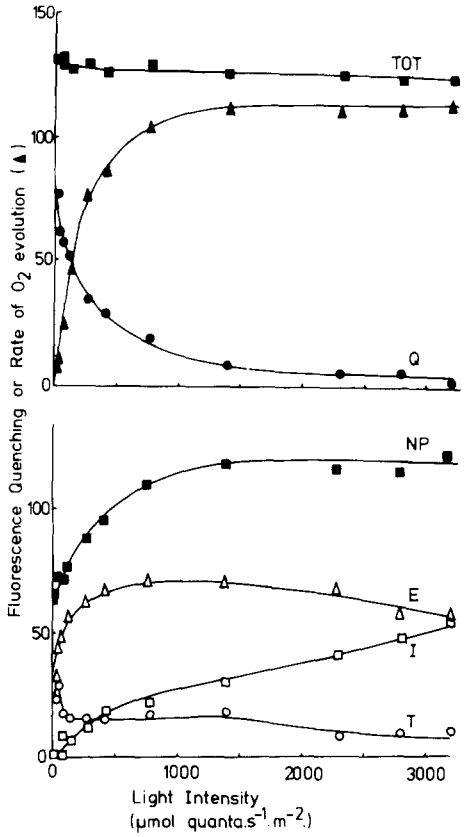

Fig. 3. Effect of light intensity on fluorescence amplitudes observed upon relief of quenching by addition of DCMU; fast rise, Q (●), slow rise, E (Δ), NaF-sensitive slow rise, T (○) and irreversible quenching I (□). Also shown is total quenching (■) and rate of O_2 evolution in μmol O_2 per mg Chl per h (▲). Experiments were performed as in Figs. 1 and 2, except that light pulsing was only used at intensities above 500 μmol quanta·s^{-1}·m^{-2}. Each point is a mean of 4–6 separate experiments.

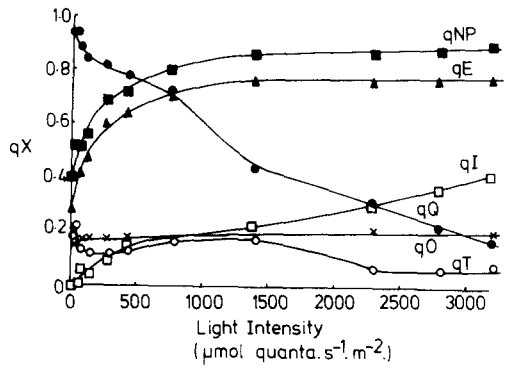

Fig. 4. Effect of light intensity on the fluorescence quenching parameters qNP (■), qQ (●), qE (▲), qT (○) and qI (□). Also show is q0, the proportional quenching of the F_0 level of fluorescence (×). For normalisation routines, see text.

In Fig. 4, the quenching amplitudes are normalised with respect to 'available' variable fluorescence, according to the principle described by Schreiber et al. [16,21,22]. The sequential relaxation of quenching upon DCMU addition allows normalisation according to the following formula: $qX = \Delta FX/(\Delta FX + FX')$, where X refers to the four quenching processes, ΔFX is the increase in amplitude of fluorescence due to the induced relaxation of quenching mechanism X, and FX' is the level of fluorescence reached after relaxation of the previous quencher minus F_0 in the order F, FQ, FE and FT, where F is the observed steady-state fluorescence, FQ ($\equiv F_m$) the level reached after a light pulse or the fast DCMU-induced rise, FE after the first slow component of the DCMU-induced rise, and FT after the completion of the slow NaF-sensitive rise. It should be noted that a quenching of F_0 was observed in these experiments as recently described [16], and this F_0 value was used in the calculation of qX. The normalised quenching values are proportional to the extents of the underlying causal processes (e.g., the proportion of closed PS II reaction centres or the size of the ΔpH). Using this procedure, several new features become apparent. The first phase in the decline in qQ as the light intensity was increased is correlated with the increase in qE which saturates with light intensity much like the rate of photosynthetic O_2 evolution. The continued fall in qQ at light intensities above saturation is not accompanied by a change in rate of O_2 evolution, and is seen to be associated with the increase in the qI component.

In the above experiments the inhibitors DCMU, NaF and chloramphenicol have allowed resolution of non-photochemical quenching. Though useful for in vitro systems this technique is obviously not applicable for use in the analysis of fluorescence quenching in whole leaves. Therefore, attempts were made to resolve the relaxation kinetics observed without the addition of DCMU and the results are presented in Figs. 5 and 6. In this experiment actinic light was turned off at steady state and saturation pulsing was continued at a slower frequency of 0.07 Hz. Pulsing at this

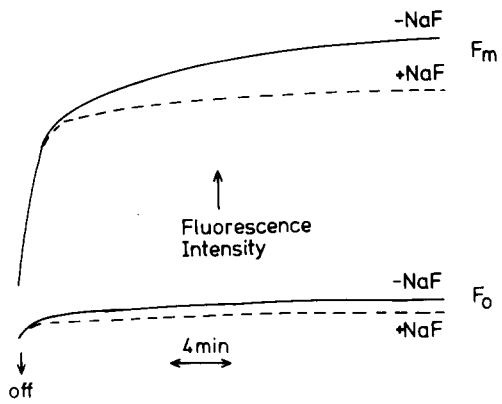

Fig. 5. Kinetics of relaxation of fluorescence quenching upon darkening (↓) after steady-state quenching had been reached in 780 μmol quanta·s^{-1}·m^{-2}. Also shown are recording made with 50 mM NaF added 30 s prior to darkening. F_m, defined here as the fluorescence level obtained in a saturating pulse, was recorded by periodic pulsing with 1 s light given every 14 s and is indicated in the upper curves. F_0, the fluorescence level recorded in the intervening dark periods when only the measuring beam was on is indicated in the lower curves.

frequency did not give any observable alteration of the dark steady state. In this way, relaxation of quenching of both F_0 and F_m could be followed. It was observed that relaxation of F_m occurred biphasically, a fast phase with a $t_{1/2}$ = 30 s and a slower phase with a $t_{1/2}$ = 8 min (Fig. 5). A third phase relaxed with a $t_{1/2}$ of about 30 min [20] and is essentially irreversible on the time scale of Fig. 5. On the basis of these kinetics it is suggested that the three phases are due to energy-dependent, phosphorylation-dependent and photoinhibition-dependent quenching, respectively. In support of this suggestion, the irreversible phase was much greater after illumination with high light (data not shown) and the slow phase was suppressed by NaF (Fig. 5). A semi-logarithmic plot of the relaxation of quenching against time resolves into two linear phases, the slower of which is eliminated by NaF (Fig. 6). The vertical intercept gives the proportion of fluorescence due to each phase; at three different light intensities, proportion of qT (relative to qT + qE) is 29, 20 and 17%. The DCMU technique used as in Fig. 3 gave values of 34, 21 and 15% in parallel experiments. As found by Bilger and Schreiber [16], the F_0 level of fluorescence was also quenched (see (Fig. 4), although in their study no F_0 quenching was observed at low light intensity. This quenching recovers when

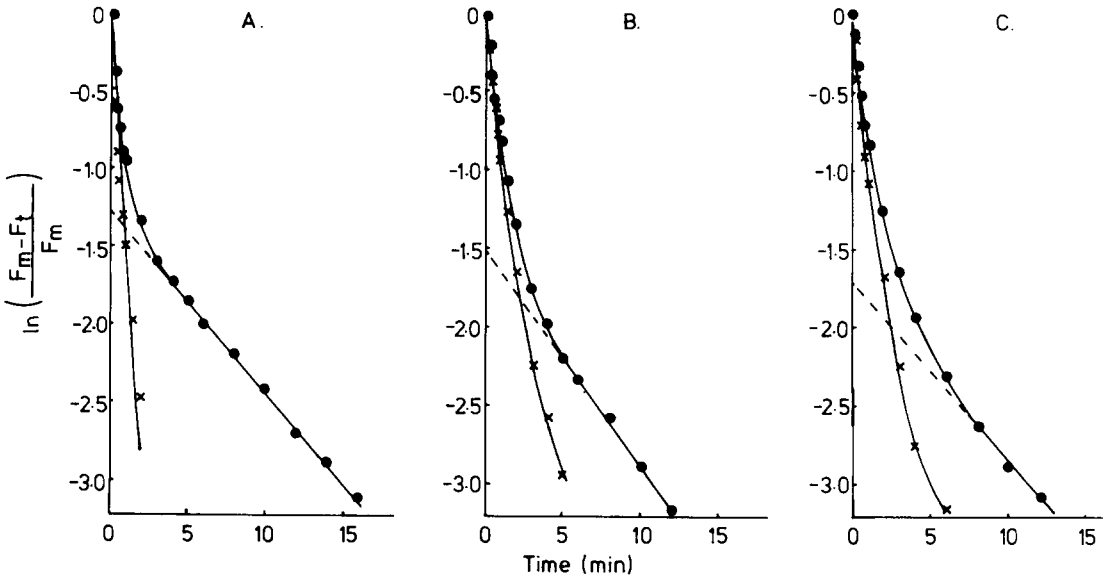

Fig. 6. Semilogarithmic plot of fluorescence relaxation observed as in Fig. 5 but recorded after illumination in 70, 1400 and 3220 μmol quanta·s^{-1}·m^{-2}. Control (●), NaF added 30 s prior to darkening (×). F_m is the fluorescence amplitude observed upon application of a light saturation pulse after complete relaxation and F_t that seen at time t after darkening.

light is turned off, the recovery also being biphasic and partially sensitive to the presence of NaF, suggesting that both qE and qT are associated with a change in F_0, and perhaps explaining the difference from the data of Bilger and Schreiber [16]. However, the magnitude of the F_0 changes are small and it is also impossible at present to ensure that true F_0 values are obtained immediately after darkening.

Discussion

The quenching of chlorophyll fluorescence has been shown to occur to an extent just over 90% of variable fluorescence at all light intensities. At light intensities approaching and above saturating for O_2 evolution, quenching is mainly non-photochemical. Non-photochemical quenching can be resolved into at least three components, based upon their relaxation times upon adding DCMU or upon darkening. Using a similar approach, Schreiber and Bilger described three phases in the relaxation of quenching upon darkening of whole leaves, although only the fast phase was identified [22]. This component with $t_{1/2} = 30$ s is the qE component, studies in isolated chloroplasts having established a strong correlation between it and the ΔpH [23]. The qE component saturates with the same characteristics as photosynthetic O_2 evolution. A slowly relaxing component of qNP which is inhibited by NaF is suggested to be due to protein phosphorylation-induced quenching, qT. This component exceeds 10% F_v only in very low light when it can be a significant proportion of qNP (43%). The fact that an NaF-sensitive component of F_0 quenching was observed is additional support for LHC II phosphorylation as the molecular mechanism for this quenching [24]. Finally, a third component, which is essentially irreversible on the time scale of the present experiments, is assigned to photoinhibition-associated quenching, qI. At light intensities above saturating, qI becomes increasingly a major fluorescence quencher, accounting for 40% of the total quenching at the highest light intensity used here. At present it is not possible to define the exact nature of qI. It has long been assumed that the quenching of fluorescence upon exposure to excess light was due to damage to Photosystem II [25]. Loss of F_v has been linked to the loss of the Q_B reaction centre polypeptide [26]. However, in experiments on isolated chloroplasts it has been shown that quenching and loss of Q_B were poorly correlated, there being an indication of two ways of decreasing the F_v/F_m ratio, an increase in F_0 due to PS II inactivation and the quenching of F_v through an effect on the pigment bed [27]. Indications of a similar separation of PS II inactivation and quenching were observed by Demmig and Björkman [28] and in recent work a strong correlation was found between quenching and zeaxanthin formation [29]. This quenching reversed with a $t_{1/2}$ of approx. 30 min, although more extreme treatments caused a further quenching that showed neglegible recovery. Clearly, qI is itself heterogeneous and further work will be required to resolve the qI observed in the present study. Examination of the data in Fig. 4 suggests two components to the rise in qI with light intensity, which may be related to this proposed heterogeneity. Furthermore, Fig. 3 shows that the light-saturated rate of photosynthesis is not inhibited, even at the highest levels of qI; a recent study of photoinhibition in barley protoplasts indicated that, although minor levels of photoinhibition only lower the quantum yield for O_2 evolution, the light-saturated rate is decreased when extensive photoinhibitory quenching occurs [20]. This suggests that at least part of the qI observed here is perhaps due to the zeaxanthin phenomenon.

The relationships between these quenching processes and the rate and efficiency of photosynthesis are complex. It clearly takes much more light to saturate qQ than the rate of O_2 evolution, perhaps an indication that other pathways of electron transfer away from Q are contributing to qQ in addition to linear transport to NADP. Either, Mehler reaction or a PS II cycle would influence qQ, but not the measured rate of O_2 evolution. The step-wise decline in qQ with increasing light intensity has also been observed by Schreiber and Bilger [22]. It is also significant perhaps that the fall in qQ in high light provides a good correlation with the rise in qI indicating that the mechanisms keeping Q_A oxidised are instrumental in preventing the development of qI.

The NaF-sensitive, slowly relaxing phase that is tentatively ascribed to protein phosphorylation is

maximum at the lowest light intensity used and declines in moderate light levels. It is unexpected to see the qT maximum at light intensities which cause negligible reduction of PS II acceptors. Work with isolated thylakoids demonstrated a strong correlation between protein kinase activity and the redox state of plastoquinone [30]. However, it has recently been shown that antimycin A can cause a marked stimulation of protein phosphorylation, suggesting that redox activation involves a component removed from PS II and possibly associated with the cytochrome b_{563}-f complex [13]. The decline in qT at higher light intensity is correlated with a rise in qE and this may well provide an explanation, since thylakoid energisation has been shown to be associated with a suppression of protein phosphorylation in isolated chloroplasts [10,13,31]. The further decline in qT as the light is raised is associated with increasing extents of qI. This observation agrees with the finding that dephosphorylation of thylakoids is induced by photoinhibitory treatment [26,32]. These data indicate that protein phosphorylation is primarily involved in increasing efficiency under light-limiting conditions, rather than in the protection against photoinhibition [33].

In recent work, the parameter qQ has been used as a measure of the fraction of open reaction centres of PS II and compared to the quantum yield of photosynthesis at each light intensity [11]. The data of Fig. 3 are plotted in this way in Fig. 7. A hyperbolic relationship is observed which passes through zero. It should be added that, because of energy transfer between reaction centres, qQ is not a linear indicator of the fraction of open reaction centres. However, since fluorescence and electron transfer are both measures of excitation dissipation, qQ and quantum yield should be linearly related; simulation of the Butler fluorescence model indeed shows that at any value of the fraction of open reaction centres, qQ and quantum yield are directly proportional, showing that the deviation from linearity in Fig. 7 cannot be due to this simple "artefact". In fact, the term "fraction of open reaction centres", as used here, is only an operational quantity refering to the relative proportions of excitation being dissipated photochemically. It has therefore been suggested

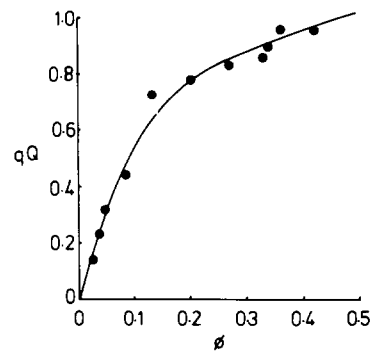

Fig. 7. Relationship between quantum yield of O_2 evolution (Φ) and qQ recorded at different light intensities. Φ is the ratio of the rate of O_2 evolution to the incident light intensity in arbitrary units.

that the deviation from linearity is due by a decline in quantum yield of PS II reaction centres that is not due to accumulation of reduced Q, but to a decrease in their intrinsic photochemical efficiency [11,34]. As the light intensity rises the quantum yield declines, as expected from the usual shape of the light saturation curve and being caused by an increased proportion of closed reaction centres; however, it is also clear that the quantum yield of open centres decreases by over 50% between 0 and 1000 μmol quanta $\cdot s^{-1} \cdot m^{-2}$ (Fig. 8). The effect of this is to keep a larger proportion of reaction centres open than would be predicted from the light-saturation curve for pho-

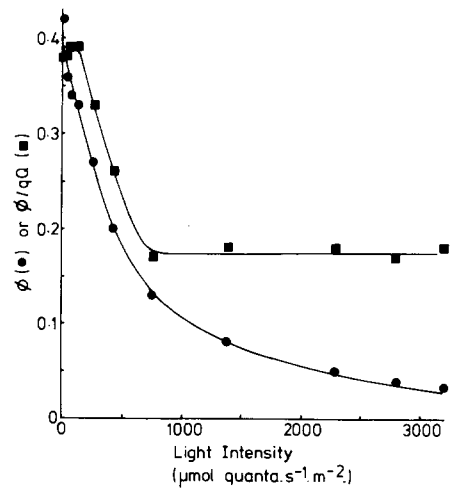

Fig. 8. Decline of quantum yield (●) and quantum yield of open reaction centres, Φ/qQ (■) at increasing light intensity.

tosynthetic O_2 evolution, hence explaining the small change in qQ observed between 0 and 1000 μmol quanta $\cdot s^{-1} \cdot m^{-2}$ in Fig. 4.

It has been suggested that this decrease in photochemical efficiency of PS II is due to the occurrence of energy-dependent quenching, qE [11,34]. In Fig. 9 the quantum yield of open centres is plotted against qE. Three phases can be identified; between zero and 0.5 qE little decrease in quantum yield occurs. Above 0.50, strong dependency is seen, the quantum yield decreasing until a maximum qE of 0.8 is reached. At light intensities above those necessary to saturate qE further increase in qNP does not cause a decline in the quantum yield of open centres (see Fig. 8). It should be added that the values for qQ at high light intensity will be highly dependent on the accuracy of the F_0 determination, and the increased scatter observed is to be expected. Data from Krause and Laasch reveal constancy of the quantum yield of open centres at different light intensity in isolated spinach chloroplasts [35], rather like the first phase in Fig. 9. In contrast, Weis, Berry and Ball [11,34] described a linear dependency that gave an intercept indicating that the quantum yield of PS II centres in the quenched state was about 25–50% of those not quenched. Such a response strongly resembles the middle phase in Fig. 9. The data here indicate that PS II centres quenched by a maximum qE have a quantum yield approx. 50% of that in the absence of qE. The present data confirm that it is the energy-dependent qE and not another component of qNP that is associated with this decrease in the quantum yield; photoinhibitory quenching presumably either completely inactivates the PS II reaction centre or quenches in the antenna and therefore would not affect the Φ/qQ value. The analysis of the kind presented in Figs. 7–9 is complicated by the presence of heterogeneity in the quenching properties of PS II [36]. It cannot be ruled out at this stage that the observed changes in quantum yield are the result of two populations of PS II, one with high quantum yield saturating in low light and another of lower quantum yield saturating in high light. A more attractive possibility is that thylakoid energisation results in a transition between two PS II types, one a high quantum yield, high fluorescence yield state predominant at low ΔpH, the other with a low quantum yield and low fluorescence yield that is formed at high ΔpH [34,37].

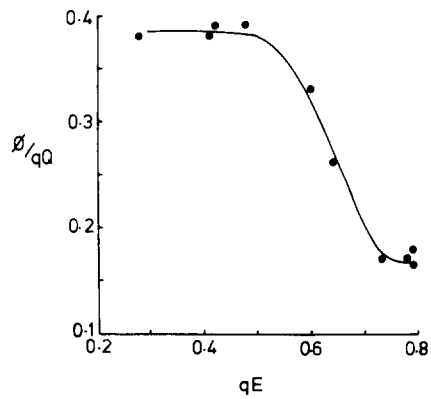

Fig. 9. Dependency of the quantum yield of open centres Φ/qQ upon the extent of energy-dependent quenching, qE, recorded at different light intensities.

The data presented here provide strong support for the rationale that non-photochemical quenching is indicative of regulatory mechanisms in the thylakoid membrane [10,37]. When the light level is raised above that necessary for photosynthesis, photochemical efficiency decreses as a result of excitation dissipation. Thus, the reaction centres do not merely passively respond to their increased rate of excitation, but respond in an active manner, decreasing their quantum yield as the light intensity increases. The function of this process may be viewed as a protective mechanism to prevent photo-damage [12]. Equally, the restriction of PS II activity may well be a way of controlling the redox poise of the intersystem carriers so that cyclic electron transport is not inhibited by over-reduction. The fact that qE is dependent on the transthylakoid ΔpH will mean that connection is established between PS II activity and photosynthetic carbon assimilation [10,11,34,35].

The contribution of photoinhibition-associated quenching to the non-photochemical quenching in high light suggests that this process, like qE, is an adaptive and useful dissipative process [10,28]. Increases in light intensity will elicit a series of responses from the chloroplast-increased photosynthesis (qQ), increased qE-induced dissipation, protein phosphorylation, photoinhibition and fi-

nally change in membrane composition. Each has different strengths of control, different time constants, different cost (e.g. in terms of lost photosynthesis when the light intensity is lowered) and the extent of usage would depend on the extent and duration of the light change. Equally, it would be predicted that different plant material (e.g., grown at different light intensities or different species) would differ in their control over excitation dissipation [10,28,37]. Finally, it is perhaps possible to speculate that the changes in PS II function that are apparent as the light intensity is raised are related to the phenomenon of PS II heterogeneity [36].

Acknowledgements

This work was supported in part by the SERC. We wish to thank Bob Furbank, Kevin Oxborough, Debbie Rees and Julie Scholes for discussion.

References

1 Walker, D.A. (1976) in Topics in Photosynthesis, Vol. 1. The Intact Chloroplast (Barber, J., ed.), pp. 235–278, Elsevier, Amsterdam.
2 Horton, P. (1985) in Topics in Photosynthesis, Vol. 6. Photosynthetic Mechanisms and the Environment (Barber, J. and Baker, N.R., eds.), pp. 135–187, Elsevier, Amsterdam.
3 Baker, N.R. and Horton, P. (1987) in Topics in Photosynthesis. Vol. 9, Photoinhibition (Kyle, D. and Osmond, B., eds.), pp. 145–168, Elsevier, Amsterdam.
4 Bradbury, M. and Baker, N.R. (1981) Biochim. Biophys. Acta 635, 542–551.
5 Krause, G.H., Vernotte, C. and Briantais, J.-M. (1982) Biochim. Biophys. Acta 679, 116–124.
6 Quick, W.P. and Horton, P. (1984) Proc. R. Soc. Lond. B 220, 361–370.
7 Quick, W.P. and Horton, P. (1984) Proc. R. Soc. Lond. B 220, 371–382.
8 Quick, W.P. and Horton, P. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 413–416, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht.
9 Quick, W.P. and Horton, P. (1986) Biochim. Biophys. Acta 849, 1–6.
10 Horton, P. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 681–688, Martinus Nijhoff, Dordrecht.
11 Weis, E., Berry, J. and Ball, T. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 553–586, Martinus Nijhoff, Dordrecht.
12 Krause, H. and Behrend, U. (1986) FEBS Lett. 200, 298–302.
13 Oxborough, K., Lee, P. and Horton, P. (1987) FEBS Lett. 221, 211–214.
14 Horton, P. (1983) Proc. R. Soc. Lond. B217, 405–416.
15 Ogren, E. and Baker, N.R. (1985) Plant Cell Environ. 8, 539–547.
16 Bilger, W. and Schreiber, U. (1986) Photosyn. Res. 10, 303–308.
17 Black, M.T., Foyer, C.H. and Horton, P. (1984) Biochim. Biophys. Acta 767, 557–562.
18 Bennett, J. (1980) Eur. J. Biochem. 104, 85–89.
19 Quick, W.P. (1986) Ph.D. Thesis, University of Sheffield.
20 Horton, P., Lee, P. and Hague, A. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. IV, pp. 59–62, Martinus Nijhoff, Dordrecht.
21 Schreiber, U., Schliwa, U. and Bilger, W. (1986) Photosyn. Res. 10, 51–62.
22 Schreiber, U. and Bilger, W. (1987) in Plant Response to Stress (Tenjunen, J., ed.), Springer, Berlin, in press.
23 Krause, G.H., Briantais, J.-M. and Vernotte, C. (1983) Biochim. Biophys. Acta 723, 169–175.
24 Horton, P. and Black, M.T. (1981) Biochim. Biophys. Acta 623, 53–62.
25 Powles, S.B. (1984) Annu. Rev. Plant Physiol. 35, 15–44.
26 Kyle, D.J. (1985) Photochem. PhotoBiol. 41, 107–116.
27 Bradbury, M. and Baker, N.R. (1986) Plant Cell Environ. 9, 289–297.
28 Demmig, B. and Björkman, O. (1987) Planta 171, 171–184.
29 Demmig, B., Winter, K., Kruger, A. and Czgan, F.-C. (1987) Plant Physiol. 84, 218–224.
30 Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) FEBS Lett. 125, 193–196.
31 Fernyhough, P., Foyer, C.H. and Horton, P. (1984) FEBS Lett. 176, 133–138.
32 Demmig, B., Cleland, R. and Björkman, O. (1987) Planta, in press.
33 Horton, P. and Lee, P. (1985) Planta 165, 37–42.
34 Weis, E. and Berry, J. (!987) Biochim. Biophys. Acta 894, 198–208.
35 Krause, G.A. and Laasch (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. IV, pp. 19–26, Martinus Nijhoff, Dordrecht.
36 Black, M.T., Brearley, T.H. and Horton, P. (1986) Photosyn. Res. 8, 193–207.
37 Horton, P., Oxborough, K., Rees, D. and Scholes, J.D. (1988) Plant Physiol. Biochem., in press.