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Uncoupler titration of energy-dependent chlorophyll fluorescence quenching and Photosystem II Photochemical yield in intact pea chloroplasts

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An investigation into how the photochemical yield of Photosystem II (Φ_p = electron transport rate / (light intensity · coefficient for photochemical quenching of chlorophyll fluorescence)) and energy-dependent quenching of chlorophyll fluorescence (qE) depend upon the transthylakoid pH gradient (ΔpH) was carried out using intact pea chloroplasts. Room-temperature chlorophyll fluorescence, 9-aminoacridine fluorescence, and oxygen uptake were measured simultaneously during titrations against nigericin, in the presence of methyl viologen. Photochemical chlorophyll fluorescence quenching (qQ) and qE were measured by the technique of DCMU addition. Titrations revealed a consistent dependence of both Φ_p and qE upon ΔpH, estimated by 9-aminoacridine fluorescence quenching (q9-aa), at three widely differing light intensities. However, whereas a threshold value of q9-aa was required in order to observe qE, Φ_p was seen to decrease at much lower values of q9-aa. The relationship between Φ_p and q9-aa during titrations against nigericin was affected only marginally by the presence of antimycin A concentrations which markedly inhibited qE. A plot of Φ_p against qE gave a relationship inconsistent with the two-state model of Weis and Berry (Biochim. Biophys. Acta 894 (1987) 198–208). The data support the existence of another mechanism, such as an electron cycle around Photosystem II, which can operate to reduce the efficiency of linear electron transport through Photosystem II.

Introduction

The efficiency of light utilisation in photosynthesis, or quantum yield (Φ_s), decreases as the intensity of illumination is increased. As secondary reactions become limiting, the fraction of incident light which can be used for photochemistry falls, and closure of reaction centres occurs. Weis et al. [1] showed that a yield for electron transport through Photosystem II, independent of the rate-limitations of subsequent reactions, can be calculated using the measured coefficient for photochemical quenching of room-temperature chlorophyll fluorescence, qQ. qQ can be taken as a measure of that fraction of incident light captured by the light-harvesting pigments associated with PS II which is actually used by PS II in photochemistry. Division of Φ_s by qQ gives the photochemical or 'intrinsic' yield of PS II, Φ_p.

It has been shown in leaves that Φ_p is not constant but decreases as the light intensity is increased [1,2]. This decrease has been correlated with an increase in non-photochemical quenching of chlorophyll fluorescence, qNP [1–4], the major part of which, under most conditions, can be ascribed to radiationless decay of excited singlet chlorophyll associated with the formation of a transthylakoid ΔpH (qE quenching [5–7]). It is argued that such dissipation of excitation energy acts to produce both a lowered efficiency of electron transport through PS II and a decreased yield of chlorophyll fluorescence. Weis and Berry [2] considered that this process operated at the level of P680 and that the decrease in Φ_p and increase in qNP resulted from the conversion of reaction centres from an unquenched, high-yield state to a quenched state of low yield. This

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_0, fluorescence level when all PSII centres are open, PS II, Photosystem II; qQ, coefficient for photochemical chlorophyll fluorescence quenching; qE, energy-dependent quenching of chlorophyll fluorescence; qNP, non-photochemical quenching of chlorophyll fluorescence; a9-aa, % quenching of 9-aminoacridine fluorescence; Chl, chlorophyll; Q_A and Q_B, primary and secondary stable electron acceptors of Photosystem II; Φ_s, rate of electron transport/light intensity; Φ_p, Φ_s/qQ; ΔpH, pH difference between the intrathylakoid space and the external medium.

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model predicts a linear relationship between Φ_p and non-photochemical fluorescence quenching: extrapolation of the line to a qNP value of 1.0 gives the Φ_p value of the quenched state, since, according to the dictates of the model, all centres are in the quenched state at qNP = 1.0 [2].

Also in leaves, Genty et al. [4] obtained data which are consistent with the idea that increased dissipation of excitation energy responsible for the quenching of fluorescence also causes the decrease in Φ_p. However, consideration of theoretical quantitative relationships between photochemical efficiency, energy dissipation, and fluorescence [8] reveals that their data is consistent with a dissipative process in the light-harvesting and/or antenna pigments associated with PS II rather than at the reaction centre [4].

Work with isolated chloroplasts has confirmed the hypothesis that the decrease in Φ_p is associated with an increase in ΔpH; removal of ΔpH with uncouplers prevents the light-intensity-dependent decrease in Φ_p [9–13]. However, some of these studies have produced evidence suggesting that the decrease in Φ_p in coupled chloroplasts is not simply the result of an increase in qE. Similar changes in Φ_p were observed in the presence or absence of concentrations of antimycin A which completely inhibit qE without affecting ΔpH [11]. Under conditions where qE is small or negligible, large decreases in Φ_p are still observed as ΔpH builds up [12,13]. Such observations, together with the failure to obtain relationships between Φ_p and qE which are consistent with the model of Weis and Berry [2], have led to suggestions that a cycle or back-reaction in the reaction centre could be operating to reduce the efficiency of electron transport through PS II, and that it is this mechanism, rather than qE, which is chiefly responsible for the observed large decreases in Φ_p [11–13].

In an attempt to resolve this question, we have investigated, using intact pea chloroplasts carrying out electron transport to methyl viologen, how Φ_p and qE depend on the size of ΔpH during titrations against the ionophoric uncoupler nigericin at constant light intensity. Clearly, if changes in qE and Φ_p are manifestations of the same ΔpH-induced process, then they will be observed to occur over the same ΔpH range.

Materials and Methods

Pea seeds (var. Kelvedon Wonder) were germinated in trays of vermiculite under fluorescent strips providing approx. 100 μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}. 2 days after first emergence (7 days after sowing), trays were placed under Wotan HQI-E mercury vapour lamps which provided a light intensity of 400 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} at the leaf surface. The illumination regime was 16 h light/8 h dark and the temperature was 18–20 °C. Plants were watered daily.

12–14 day old plants were darkened for 24 h prior to use. Intact chloroplasts were prepared according to the method of Cerovic and Plesnicar [14]. The resuspension medium contained 330 mM sorbitol, 50 mM Hepes, 10 mM KCl, 1 mM EDTA, 2.5 mM $MgCl_2$, and bovine serum albumin (20 mg $\cdot$ ml^{-1}) at a pH of 7.9. All chloroplast preparations were between 75 and 90% intact, according to the ferricyanide method [15]. Chloroplasts were stored on ice for one hour, prior to use.

Chlorophyll fluorescence, 9-aminoacridine fluorescence, and oxygen uptake were measured simultaneously using similar apparatus to that described in Ref. 16. The illumination chamber (Hansatech) had four ports, at intervals of 90°. Actinic illumination of variable intensity from a Schott lamp (red light) and the chlorophyll fluorescence measuring beam (from a Walz fluorimeter) were passed down a fibre-optic to a port 180° from the port through which the 9-aminoacridine fluorescence excitation beam entered (50 W tungsten source from a Volpi 250HL modulated at 170 Hz and passed through Corning 5-58 and 7-39 glass filters). Another port at 90° to each of these ports allowed 9-aminoacridine fluorescence (defined by 480 nm shortpass, 450 nm broad-band, and 457.9 nm narrow-band interference filters, and two Corning 4-96 glass filters) to pass to a photomultiplier tube connected to a Bentham lock-in amplifier. The remaining port allowed delivery of saturating red light (greater than 5000 μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}) from a Volpi 250HL through a Uniblitz shutter. Signal outputs from the chlorophyll fluorimeter, lock-in amplifier, and oxygen differentiator were fed to a multi-pen chart recorder.

The assay medium contained 330 mM sorbitol, 50 mM Hepes, 10 mM KCl, 1 mM EDTA, 2.5 mM $MgCl_2$, 0.1 mM methyl viologen, 1 mM NaN_3, 2 μM 9-aminoacridine at a pH of 7.9. Nigericin was added to the indicated concentration. Chloroplasts were then added to a concentration of 25 μg $\cdot$ ml^{-1}; the final volume was 1.5 ml. The concentration of ethanol was always less than 1% of this volume.

The F_0 level of fluorescence was measured using the low-frequency measuring beam (1.6 kHz; light intensity, 0.25 μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}) of the fluorimeter; after 1 min, the actinic light was turned on and the measuring beam switched to the higher frequency (100 kHz) to give a cleaner signal. After 2 min illumination, the chart speed was increased and 50 μM DCMU added. Saturating light was applied as DCMU was added and continued until the fast phase of the DCMU-induced fluorescence rise was complete (1–2 s); the slow phase was allowed to take place in the presence of actinic light only. Chlorophyll fluorescence quenching coefficients were calculated as in Ref. 6. The coefficient for 9-aminoacridine fluorescence quenching, q9-aa, refers to

the percentage of total fluorescence which was quenched after 2 min light. All rates of oxygen uptake refer to values after 2 min illumination. All light intensities were measured inside the chamber.

Results

Fig. 1 shows traces copied directly from chart paper, showing 9-aminoacridine fluorescence, differentiated oxygen signals, and chlorophyll fluorescence measured simultaneous in intact pea chloroplasts in the presence of different nigericin concentrations. Addition of DCMU to coupled chloroplasts in the light (Fig. 1, bottom left) causes chlorophyll fluorescence to rise in a biphasic manner: the fast phase represents the removal of photochemical quenching (qQ), while the slow phase reflects removal of that quenching which is associated with the transthylakoid pH gradient (qE) [6]. The latter effect of DCMU arises through its inhibition of linear electron transport: it is important to note that under

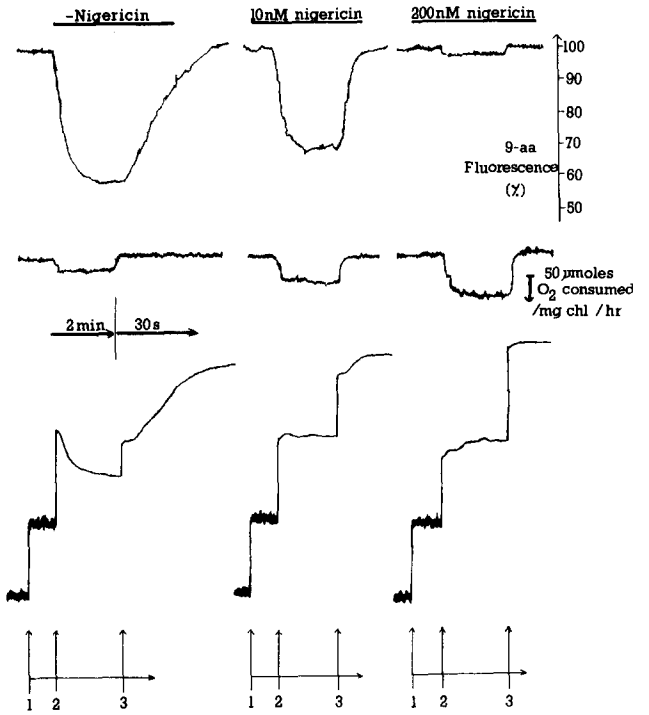

Fig. 1. Copy of traces showing 9-aminoacridine fluorescence quenching (top), differentiated rates of oxygen consumption (middle), and chlorophyll fluorescence (bottom), for intact chloroplasts in the presence of zero (left), 10 nM (centre), and 200 nM (right) nigericin at a light intensity of 121 μmol quanta$\cdot m^{-2}\cdot s^{-1}$. The low-frequency, modulated chlorophyll fluorescence measuring beam was switched on at point 1; after measurement of the F_0 level of fluorescence, the actinic light was turned on at point 2, after 2 illumination 50 μM DCMU was added and additional saturating illumination applied for 1–2 s (point 3). Prior to DCMU addition, the chart speed was 1 cm$\cdot$min^{-1}; on DCMU addition, the chart-speed was increased by a factor of 5 to enable accurate resolution of photochemical and non-photochemical fluorescence quenching. The time-scale subsequent to DCMU addition is therefore 1 cm = 12 s.

TABLE I

9aa quenching, rates of oxygen uptake, and qQ at three light intensities, for intact chloroplasts in the coupled state, and in the presence of high concentrations of nigericin

All values are the mean of three chloroplast preparations; standard deviations are shown in brackets.

Light intensity (μmol quanta$\cdot m^{-2}\cdot s^{-1}$)	q9-aa (%)	O_2 uptake (μmol$\cdot m^{-2}\cdot s^{-1}$)	qQ
32 coupled	35 (1.3)	23 (2.1)	0.79 (0.01)
+100 nM nigericin	1.7 (0.3)	39 (6.6)	0.81 (0.01)
121 coupled	47 (1.5)	28 (1.6)	0.42 (0.02)
+200 nM nigericin	2.1 (0.2)	95 (8.0)	0.56 (0.08)
937 coupled	48 (1.1)	45 (1.7)	0.08 (0.01)
+1 μM nigericin	0	209 (15.4)	0.15 (0.02)

these conditions, i.e., methyl viologen as electron acceptor, addition of DCMU always caused complete dissipation of the light-induced ΔpH, as shown by complete decay of the 9-aa fluorescence quenching (Fig. 1).

The effects of inclusion of a low (Fig. 1, centre), or high (Fig. 1, right) concentration of nigericin in the assay medium, are as might be expected: the extent of 9-aa fluorescence quenching (q9-aa) decreases, while the rate of oxygen uptake and the steady-state level of chlorophyll fluorescence increases. In addition, the proportion of the fluorescence rise that occurs rapidly (within 1–2 s) upon addition of DCMU increases at the expense of the slow phase.

Table I shows the influence of light intensity on the values of these parameters either in the absence, or in the presence of a high concentration of nigericin. At all light intensities, the values of q9-aa are reduced to negligible levels by the presence of these nigericin concentrations (Table I). This is accompanied by a stimulation of both the rate of oxygen uptake and of qQ, although the extent of the stimulation varies with light intensity (see also Fig. 2).

Under our conditions, q9-aa saturates at relatively low light intensities (around 100 μmol quanta$\cdot m^{-2}\cdot s^{-1}$) (Table I). This light intensity is considerably lower than that required to saturate electron transport (cf. coupled and uncoupled rates of oxygen uptake at 121 and 937 μmol quanta$\cdot m^{-2}\cdot s^{-1}$; Table I). It has been noted before that 9-aa fluorescence quenching saturates at low light intensities (10 W$\cdot m^{-2}$) in intact chloroplasts [17]. In that study, as in this, no sink for the light-induced ΔpH existed, enabling the production of a comparatively large ΔpH at low light intensity. Thus, even at 32 μmol quanta$\cdot m^{-2}\cdot s^{-1}$, q9-aa is 75% of its maximum value. However, Fig. 2a shows that the concentrations of nigericin required to remove the quenching increases as the light intensity increases. This shows

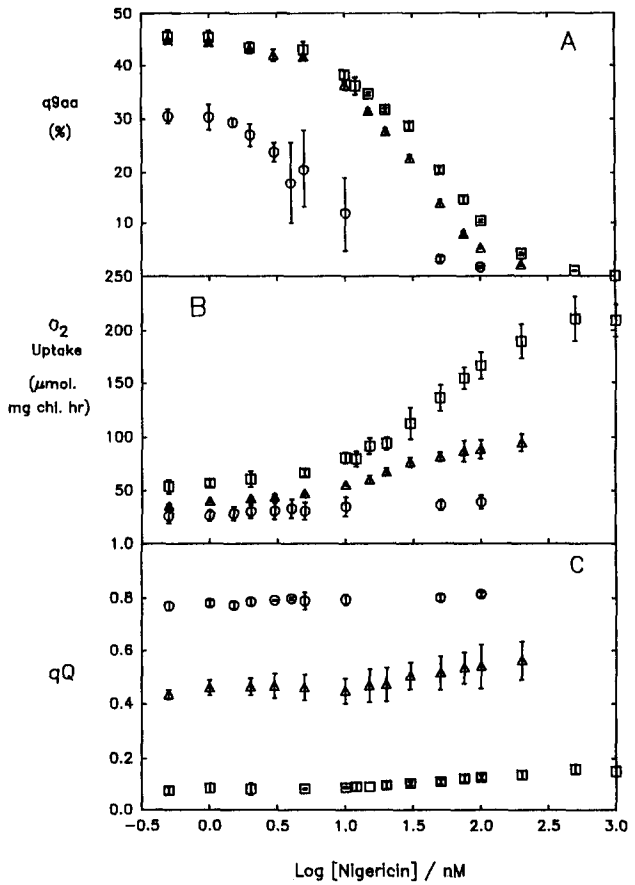

Fig. 2. 9-Aminoacridine fluorescence quenching (A), rates of oxygen uptake (B), and coefficient for photochemical quenching of chlorophyll fluorescence (C), in intact pea chloroplasts, plotted against concentration of added nigericin, for titrations at light intensities of 32 (circles), 121 (triangles), and 937 (squares) μmol quanta$\cdot m^{-2}\cdot s^{-1}$. All points are the mean of three chloroplast preparations; error bars show one standard deviation each way.

that the capacity for production of ΔpH differs markedly between these light intensities.

Fig. 2b shows the pattern of increase in the rate of oxygen uptake as the concentration of nigericin is increased. At the lowest light intensity (32 μmol quanta $\cdot$ $m^{-2}\cdot s^{-1}$), only a small stimulation of the rate is seen (by a factor of 1.6 as nigericin is increased from 0.5 nM to 100 nM). At such a light intensity, the rate of electron transport is predominantly light-limited. At 121 μmol quanta $\cdot m^{-2}\cdot s^{-1}$, rates increase by a factor of 2.7 from the lowest to the highest nigericin concentration, whereas at the highest light intensity (937 μmol quanta $\cdot$ $m^{-2}\cdot s^{-1}$), this factor rises to 3.9 (Fig. 2b).

Fig. 2c shows that the proportion of incident excitation captured by PS II which is actually used for photochemistry at PS II, represented by qQ, increases on uncoupling. A very small increase is seen at the lowest light intensity (0.77 to 0.81), and, as for oxygen uptake rates, the relative stimulation of qQ by uncoupler increases as the light intensity increases (0.43 to 0.56, and 0.08 to 0.15, at 121 and 937 μmol quanta $\cdot$ $m^{-2}\cdot s^{-1}$, respectively). It is clear from Fig. 2 that, for titrations against nigericin at all three light intensities, the rate of electron transport through PS II is stimulated by a factor markedly greater than the proportion of incident light which is actually being used by PS II in photochemistry. This means that the photochemical yield of PS II, Φ_p, is increasing as the concentration of uncoupler increases.

Fig. 3a shows how the change in Φ_p depends on ΔpH, estimated with 9-aa, using the data from the titrations against nigericin. Also shown are data obtained for coupled chloroplasts by varying light intensity. The data for Φ_p shows the same relationship with q9-aa at all three light intensities; data obtained by varying intensity of illumination of coupled chloroplasts (open symbols) also falls on the same curve. At the two highest light intensities (closed squares and triangles), Φ_p shows a decrease of approx. 60% as q9-aa increases from zero to maximal levels. The rate of this decrease, with respect to q9-aa, increases as q9-aa increases; at all light intensities, 30% of this decrease occurs before q9-aa reaches 20% (Fig. 3a).

Fig. 3b shows how qE$_2$ measured by the slow phase of quenching relaxation upon the addition of DCMU, depends upon q9-aa, during titrations against nigericin. Again the open symbols show data obtained by varying the intensity of illumination of coupled chloroplasts. There appears to be a threshold q9-aa of about 18–20%,

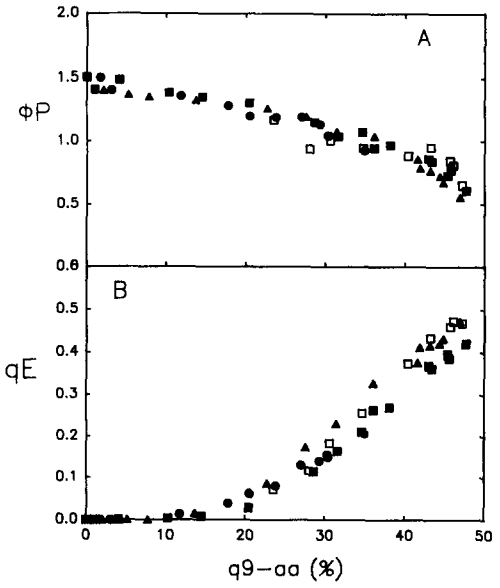

Fig. 3. Comparison of the dependence of Φ_p (A) and qE (B) upon quenching of 9-aa fluorescence for titrations against nigericin (decreasing from left to right) at light intensities of 32 (closed circles), 121 (closed triangles) and 937 (closed squares) μmol quanta$\cdot m^{-}s^{-1}$. The open symbols show data obtained for coupled chloroplasts by varying the light intensity. All points are the mean of three chloroplast preparations. Standard deviations for the qE data are not shown for reasons of diagrammatic clarity, but were typically 10% of the mean. Units for Φ_p are arbitrary.

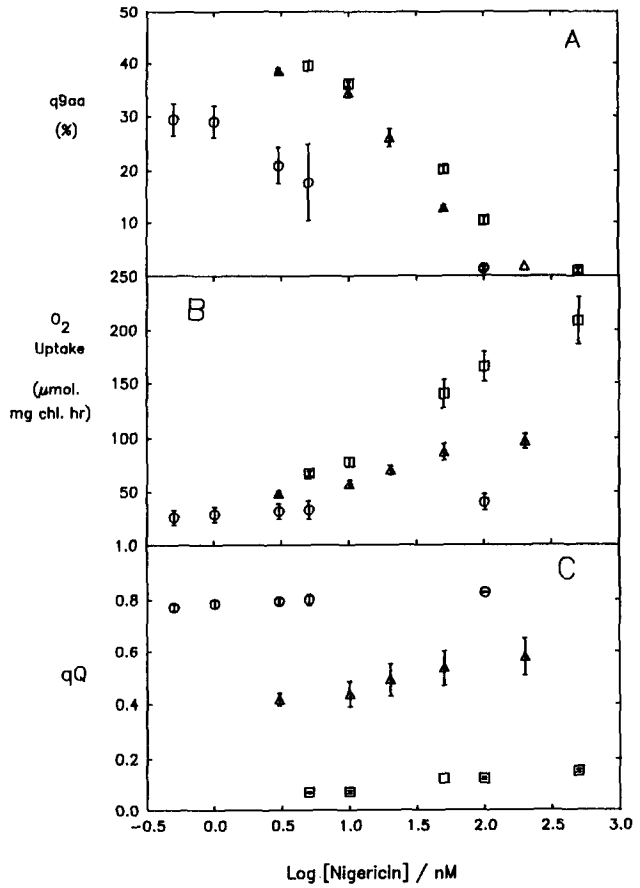

Fig. 4. 9-Aminoacridine fluorescence quenching (A), rates of oxygen uptake (B), and co-efficient for photochemical chlorophyll fluorescence quenching (C), plotted against concentration of added nigericin at three light intensites in the presence of 1 μM antimycin A. Light intensities and symbols as for Fig. 2. All points are the mean of three chloroplast preparations; error bars show one standard deviation each way.

below which qE is zero. The existence of a threshold ΔpH for qE formation has been previously noted [5,11]. After this threshold is reached, qE increases in linear proportion to q9-aa (Fig. 3b). It is clear from a comparison of Fig. 3a and b that, under our conditions, titrations of Φ_p and qE against nigericin reveal a different dependence of the two phenomena upon ΔpH.

Low concentrations of antimycin A have been shown to prevent the formation of qE, with only minor effects upon q9-aa [18]. We therefore carried out titrations of the above parameters against nigericin in the presence of 1 μM antimycin A (Fig. 4). A comparison of Fig. 4 with Fig. 2 shows that q9-aa, rates of oxygen uptake, and qQ showed similar dependence upon added nigericin concentration as for titrations in the absence of antimycin A. This is reflected in the data of Fig. 5, which show that the inclusion of antimycin A during the titrations affected the decrease in calculated Φ_p values to only a marginal extent as q9-aa increased (cf. Fig. 3a and Fig. 5a). At a q9-aa value of 45%, Φ_p values ranged from 0.7 to 0.8 in the absence of antimycin (Fig.

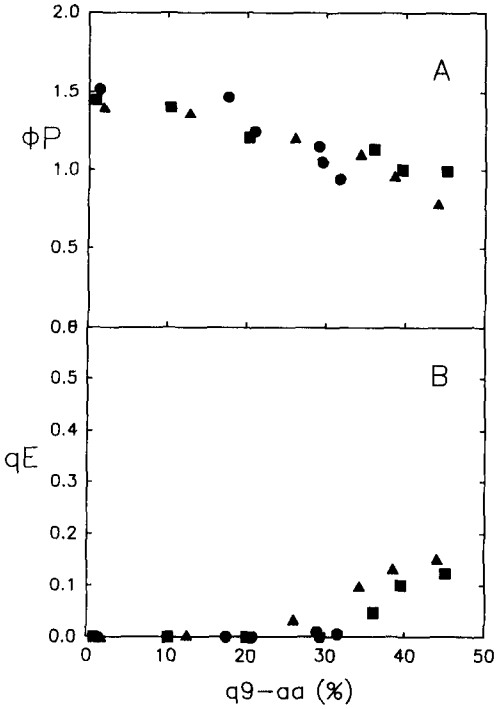

Fig. 5. Comparison of the dependence of Φ_p (A) and qE (B) upon quenching of 9-aa fluorescence for titrations (nigericin decreasing from left to right) at three light intensities in the presence of 1 μM antimycin A. Symbols as for Fig. 3. All points are the mean of three chloroplast preparations. Units for Φ_p are arbitrary.

3a), and from 0.75 to 1.0 in its presence (Fig. 5a). This minor effect is in contrast to the marked inhibition of qE by 1 μM antimycin A: at the highest light intensity significant qE was observed only above q9-aa values of 30%, and even then reached only 30% of the values of qE seen in the absence of antimycin (cf. Fig. 5b and Fig. 3b).

Fig. 6 shows a plot of Φ_p against qE using the data obtained from titrations against nigericin (in the ab-

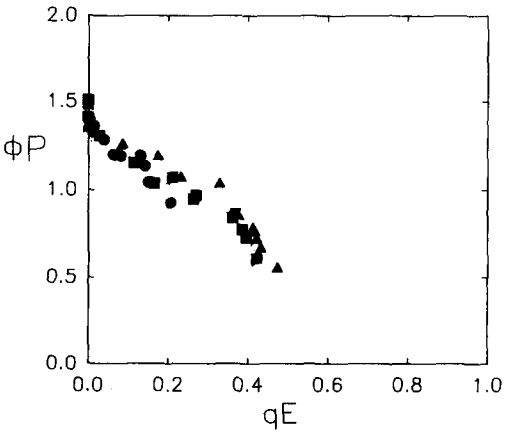

Fig. 6. Plot of data obtained from uncoupler titrations at three light intensities to show the relationship between Φ_p and qE. Light intensities at which titrations were carried out are denoted by the same symbols as for fig. 3. All points are the mean of three chloroplast preparations. Units for Φ_p are arbitrary.

sence of antimycin A). The plot uses 41 data points, each of which is the mean of three replicates, obtained at three light intensities (the lowest and the highest differing by a factor of 30). It is possible to fit the data to a straight line; such a line would intercept the qE axis at a point between 0.8 and 0.9. Equally, it could be argued that the data show a multi-phasic relationship between Φ_p and qE: certainly, the initial decrease in Φ_p which occurs while qE is zero or negligible (Fig. 6) reflects the decrease in Φ_p which occurs below q9-aa values of 20% (see Fig. 3a). It might also be considered that Fig. 6 shows a further two phases, as qE begins to build up; a relatively gentle decrease in the value of Φ_p for qE values between 0 and 0.3, followed by a steeper slope as qE exceeds 0.3. Again this reflects patterns shown in Fig. 3: whereas qE increases in direct proportion to q9-aa (Fig. 3b), the rate of decrease of Φ_p increases as q9-aa increases (Fig. 3a). This is particularly true at values of q9-aa above 40%, at which point qE is 0.3–0.35 (cf. Fig. 6).

Discussion

In this work, we have titrated intact pea chloroplasts against nigericin in order to investigate how PS II photochemical efficiency and ΔpH-dependent energy dissipation depend upon the magnitude of ΔpH. There are doubts concerning the validity of using 9-aa fluorescence as an absolute measure of ΔpH values, particularly because of the possibility of binding of 9-aa to the thylakoid membrane surface [19]; for this reason we have used 9-aa semi-quantitatively and have made no attempt to calculate actual ΔpH values. The data of Fig. 3 reveal that both Φ_p and qE show consistent relationships with q9-aa during uncoupler titrations at light intensities differing in magnitude by a factor of 30.

It is interesting that, as chloroplasts become increasingly uncoupled by titrating against nigericin at constant light intensity, the redox state of Q_A, indicated by qQ, remains relatively constant (Fig. 2c and Fig. 4c). This is particularly true at the lower light intensities. This suggests that, as ΔpH is removed, the decreasing restriction on electron transport at the plastoquinone oxidation step (tending to increased oxidation of Q_A) is counter-balanced by an increasing efficiency of electron transport through PS II (which tends to increase the extent of reduction of Q_A).

One of the problems with using variations in light intensity to study the relationship between qE and Φ_p is that, at low light intensities, the rate of electron transport is often so low as to prohibit accurate measurement. This inability to measure Φ_p at relatively low values of ΔpH may be responsible for distortion in the apparent relationship between qE and Φ_p. This problem is not encountered in these experiments, since in chloroplasts, during titrations against uncoupler at constant light intensity, the highest rates of electron transport are observed at low values of ΔpH (Fig. 2b and Fig. 4b). At these comparatively low ΔpH values (below 15–20% 9-aa quenching), qE is zero at all light intensities (Fig. 3b). Despite this, a significant fall in Φ_p is observed (Fig. 3a). Our data suggest that decreases in Φ_p begin at low light intensities, i.e., at light intensities too low to support the formation of significant qE. This hypothesis may be difficult, if not impossible, to test under in vivo conditions, because of the difficulties of measurement of electron transport rates through PS II at such light intensities.

The problems which might occur in exploring the relationship between Φ_p and qE during variations in light intensity can be appreciated by considering the data of Fig. 6. Although one might consider that Fig. 6 demonstrates a simple, linear relationship between Φ_p and qE, comparison of the data with that of Fig. 3 indicates that this is not so. The fall in Φ_p at q9-aa values too small to support the formation of any qE (Fig. 3) is reflected in the spread of Φ_p values at $q\mathrm{E} = 0$ (Fig. 6). The 'control', uncoupled, value of Φ_p is actually 1.5 at all light intensities (Fig. 3a), but, as Fig. 6 shows, values of Φ_p can vary between 1.3 and 1.5 at $q\mathrm{E} = 0$. For the data obtained by variations in the intensity of illumination of coupled chloroplasts, the mean values of qE and Φ_p are 0.07 and 1.17, respectively, at the lowest light intensity, 12.4 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$ (q9-aa value of 23%; Fig. 3, open symbols). A plot of Φ_p against qE for data obtained in this way yields a straight-line relationship, with a Φ_p value of 1.2–1.3 at $q\mathrm{E} = 0$ (not shown).

A multiphasic relationship between Φ_p and qE is incompatible with the two-state reaction centre model of Weis and Berry [2], which predicts a linear relationship between Φ_p and qE. This model also predicts that the line would extrapolate to a Φ_p value of 0 or greater, at $q\mathrm{E} = 1.0$. Thus, it can be seen that even if one maintains that the data of Fig. 6 can be described by a straight line, such a line would describe a relationship incompatible with the two-state model of Weis and Berry [2], since Φ_p would decrease to 0 while 10–20% of the centres were still unquenched (Fig. 6). Equally, the data cannot be explained solely by a quenching in the antenna and light-harvesting chlorophylls associated with PS II, as can the data of Genty et al. [4]. The small inhibition of the decrease in Φ_p co-incident with inhibition of qE by antimycin A (cf. Figs. 3 and 5) suggests that qE does exert some influence over the efficiency of electron transport through PS II. However, the different dependence of Φ_p and qE upon ΔpH (Fig. 3), and the fact that the decrease in Φ_p is mostly unaffected by antimycin A (Fig. 5), suggest that this influence is small, and that the changes in Φ_p which we observe are due, in the main, to another mechanism.

One such possible mechanism is a dissipative cycle of

electrons around, or a back-reaction within the PS II core. Electron cycling has been proposed before in order to explain various experimental observations [7,11–13,20–24], and could function physiologically as an attempt to equilibrate charge across the membrane, thereby preventing over-reduction of the quinone pool and excessive accumulation of oxidised P680, both of which have been proposed as causal factors of photoinhibition [25,26].

Lastly, it has recently been suggested [3] that artefactual data resulting from failure to use a fully saturating light-pulse in order to resolve photochemical and non-photochemical quenching may be a possible explanation of the different relationships which have been obtained between Φ_p and qE, or between Φ_p and qNP. In this context, it is perhaps worth mentioning that we have data which suggest that, under the experimental conditions used here, it may not be possible to fully reduce Q_A, using saturating light pulses: double-reciprocal plots of the pulse-induced fluorescence rise against the light intensity of the pulse produce curves which extrapolate, for infinite light intensity of the pulse, to a fluorescence peak height lower than the height of the fast phase of the DCMU-induced fluorescence rise (Noctor and Horton, unpublished results). This is particularly true for uncoupled chloroplasts, where the discrepancy may be as much as 20–30%

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