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# Use of tentoxin and nigericin to investigate the possible contribution of ΔpH to energy dissipation and the control of electron transport in spinach leaves

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**(a) Spinach leaf discs were floated overnight on various concentrations of tentoxin, an ATP synthase inhibitor, or nigericin, an uncoupler. They were then illuminated in saturating $CO_2$. (b) With tentoxin, the inhibition of photosynthesis was accompanied by lower ATP/ADP ratios and increased 'energy' quenching of chlorophyll fluorescence. There was a small increase in the reduction of the Photosystem II acceptor, $Q_A$, as monitored by photochemical quenching of chlorophyll fluorescence. However, activation of NADP-malate dehydrogenase decreased, showing that the acceptor side of Photosystem I becomes more oxidised. (c) With nigericin, the inhibition of photosynthesis was accompanied by decreased ATP/ADP ratios, decreased energy quenching, $Q_A$ became more reduced than with tentoxin, and the acceptor side of Photosystem I also became more reduced. (d) The results are used to discuss the contribution of 'energy' quenching to energy dissipation, and the operation of photosynthetic control in leaves.**

## Introduction

Studies with isolated thylakoids and chloroplasts have shown that rising ΔpH leads to a 'high energy state' quenching of chlorophyll *a* fluorescence (qE) which is taken as a measure for a pH-triggered mechanism for thermal energy dissipation at PS II [1,2]. The mechanism of quenching is still unknown, but experiments with chloroplasts show it can protect against photoinhibition [3]. Studies with thylakoids have also show that increased ΔpH will restrict the rate of electron transport, probably acting to inhibit the reoxidation of plastoquinone at the cytochrome $b_6f$ complex [4]. This 'photosynthetic control' has also been shown to operate in isolated chloroplasts [5]. However, the significance of ΔpH for energy dissipation and the control of electron transport in leaves is less well understood.

Many studies of qE have been carried out in leaves during transients, light or $CO_2$ saturation curves, or during stress (see Refs. 6–10). These measurements were usually carried out to estimate thylakoid 'energisation', in a similar way to the use of light scattering [11]. Fewer studies have explicitly addressed the probable function of energy quenching as a mechanism for energy dissipation [12,13]. Study of energy quenching in leaves is also complicated because a very large and variable part of the non-photochemical quench in leaves is due to processes other than qE [13,14]. Depending upon how the measurements have been carried out, published values may have some, little or no resemblance to the actual value of *qE*.

There have been relatively few studies of the effect of ΔpH on electron transport in leaves, because of difficulties in measuring the redox state of PS I components. Two recent studies, however, reveal that $Q_A$ may become more reduced in conditions where the non-photochemical quench increases, while PS I becomes more oxidised [15,16]. This is consistent with the operation of 'photosynthetic control' in leaves. These results were obtained over a light saturation curve, using $A_{820}$ to monitor P-700 [16] and during a comparison of $CO_2$-

Abbreviations: $A_{820}$, absorbance at 820 nm; Chl, chlorophyll; Fru-2,6-$P_2$, fructose-2,6-bisphosphate; NADP-MDH, NADP-dependent malate dehydrogenase; ΔpH, transthylakoid pH gradient; $P_i$, inorganic phosphate; PGA, 3-phosphoglyceric acid; PS I, Photosystem I; PS II, Photosystem II; *qE*, value for 'energy'-dependent quenching; *qNP*, value for non-photochemical quenching; *qQ*, value for photochemical quenching; triose-*P*, sum of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.

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saturated and $CO_2$-limited photosynthesis, using the activation state of NADP-MDH as an indicator for the redox state of PS I acceptor side [16].

Tentoxin is a specific inhibitor of the chloroplast ATP synthase [17,18], which has no effects on mitochondrial ATP synthesis [17]. Evidence for its operation in vivo is provided by studies showing a direct correlation between tentoxin toxicity and the susceptibility of the isolated $CF_1$-ATPase to tentoxin inhibition in different plants [19,20]. Tentoxin has been widely used to study the $CF_1$-ATPase (see Refs. 21–23). In the following experiments we have manipulated the thylakoid $\Delta pH$ in leaves by adding tentoxin to inhibit ATP synthase and generate a large $\Delta pH$, or by adding uncoupler to dissipate the thylakoid $\Delta pH$. We have investigated the effect on $CO_2$-saturated photosynthesis, on $Q_A$ reduction, on activation of NADP-MDH, and on energy-quenching.

## Methods

Spinach was grown in conditions of controlled light (450 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 9 h photoperiod), temperature (27 °C light, 18 °C dark) and humidity (30%) in hydroponic culture as in Refs. 9 and 16. Leaf discs (11 mm diameter) were cut 2 h after the end of the photoperiod, and were floated overnight on water containing tentoxin or nigericin, as specified in the legend. Preliminary experiments showed that leaf discs floated overnight and leaf discs taken directly from plants at the end of the dark period had similar rates of photosynthesis, partitioning between sucrose and starch, and metabolite levels (data not shown).

Photosynthesis was measured in a leaf disc $O_2$ electrode (Hansatech, Kings Lynn, England) at 15 °C in saturating $CO_2$ [9,16]. The $O_2$ electrode was calibrated by comparing the signal at 21% $O_2$ and in $N_2$, and also by introducing 1-ml aliquots of air into the closed chamber. The latter approach also provided the volume of the chamber (5.2 ml). The rates of photosynthesis were related either to chlorophyll, or to leaf area, using the empirical relation that 1 $m^2$ of leaf contained 516 ± 5.5 mg Chl. The rates estimated from the $O_2$ electrode in saturating light and $CO_2$ (about 28 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were similar to those reported previously from infra red gas analysis measurements of $CO_2$ uptake with spinach grown in similar conditions and then allowed to photosynthesis in saturating light and $CO_2$ [24].

Chlorophyll fluorescence was measured using a PAM chlorophyll fluorescence measuring unit (Heinz Walz, Effeltrich, F.R.G.) as in Ref. 7 modified as in Refs. 9 and 16. Light was delivered via a fibre optic from a Schott KL 1500 light source. The actinic light was supplemented by 700 ms pulses of super-saturating light (3600 $\mu mol \cdot m^{-2} \cdot s^{-1}$). If the pulse was shortened to 500 ms, the fluorescence yield was decreased. The fluorescence signal was not increased if the pulse was increased to 1 or 2 s. Values for photochemical quenching ($qQ$) were estimated as in Ref. 7, taking care to use the $F_0$ value obtained immediately after turning the actinic light off. Values for $qNP$ and $qE$ were estimated using a formula $q = (F_s - F_v)(F_s - F_0)$, where $F_v$ is the variable fluorescence in actinic light and $F_s$ is the fluorescence yield during a 700 ms supersaturating pulse applied to 12 h dark-adapted leaves (to estimate $qNP$) or during a super-saturating 700 ms pulse applied 1.5–2.5 min after removing the actinic light (to estimate $qE$). The longer time interval was used in samples which were inhibited with tentoxin, and was selected by reference to the rise of $F_0$, which provides a continuous qualitative estimate for the decay of $qE$.

Leaves were transferred to liquid $N_2$ and quenched under continued illumination at the light intensity used in the leaf disc electrode [9,16]. The extraction and assay of NADP-MDH, the correction for a low non-specific contribution from NADH-MDH, and the expression of the activation as a fraction of the total fully activated enzyme was carried out exactly as in Ref. 16. Extracts were prepared in 10% (v/v) $HClO_4$ and assayed for adenine nucleotides as in Ref. 25. Extracts were prepared in $CH_3OH/CHCl_3$ and assayed for phosphorylated intermediates and Fru-2,6-$P_2$ as in Refs. 9 and 16 using a ZFP 22 dual-wavelength photometer (Sigma, Berlin).

The rate of electron transport, $J_e$, was estimated as the net $O_2$ evolution plus the rate of dark respiration in dark-adapted leaves (about 3 $\mu mol\ O_2 \cdot m^{-2} \cdot s^{-1}$). It was assumed that photorespiration was negligible in these conditions (400 $\mu$l 2 M bicarbonate buffer (pH 9.3) distributed on a felt mat with an area of 5.5 cm) because no burst of $O_2$ uptake associated with photorespiration was found after darkening. The rate of electron transport was expressed as $\mu mol\ O_2$ released per $m^2$ per s; and must be multiplied by 4 to give the net flux of electrons through PS II. Apparent quantum yield ($\Phi_S$) was estimate as $J_e/I$, where $I$ is the incident light intensity. Photochemical yield ($\Phi_P$) was estimated as $\Phi_S/qQ$ [12].

## Results

*Determination of qE, qQ and NADP-MDH activation*

The approach taken in these experiments depends on measurement of the redox state of the acceptor sides of PS I and PS II and on the measurement of qE as a qualitative indicator of thylakoid energisation. The activation state of NADP-MDH was taken as a metabolic indicator for the redox state of the stromal NADPH system and, hence, the acceptor side of PS I [16]. Reductive activation of NADP-MDH via the thioredoxin system is strongly modulated by NADP [26] and the activation of the enzyme correlates strongly

with the stromal NADPH/NADP ratio [27]. Activation is expressed as a fraction of the activity found after fully activating NADP-MDH in vitro, providing a value which can be compared in different experiments. Activation approaches zero in the dark and increases up to about 60% under 21% $O_2$ at 15°C in saturating light and $CO_2$ [16].

The redox state of the PS II acceptor ($Q_A$) was monitored by measuring photochemical quenching ($qQ$). Leaves were illuminated for 10–15 min with actinic light supplemented with flashes (0.7 s, 3600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) every 30 s, to reduce temporarily the PS II acceptor ($Q_A$). The decrease of fluorescence quenching during these pulses can be used to estimate the proportion of PS II centres with open traps (i.e., oxidised $Q_A$) [7]; the $F_0$ value for this calculation was obtained by stopping the flashes, and then darkening the leaf 20 s later. When $qE$ is high, the $F_0$ value can decrease by 25%, compared to $F_0$ in predarkened leaves (see also Ref. 12).

The non-photochemical quench represents that portion of the fluorescence quenching which is not reversed by these saturating light pulses [7]. In all our experiments, leaf material was used after at least 12 h dark adaptation to ensure that all slow-relaxing components of the non-photochemical quench had been reversed. The total non-photochemical quench was estimated with reference to the fluorescence yield of the first supersaturating pulse after this dark adaption. The non-photochemical quench includes energy-dependent quenching, but it also includes additional components [7,14,28] which can make a large and varying contribution to the total non-photochemical quench [13,14]. The contribution of qE can be estimated because it relaxes rapidly ($t_{1/2} = 50–60$ s) after darkening, and this relaxation is accompanied by a marked rise of $F_0$ back to a value which is close to that found in pre-darkened leaves (data not shown). To estimate qE in the following experiments, leaves were darkened for 1.5 min, and then given a 700 ms flash (3600 $\mu mol \cdot m^{-2} \cdot s^{-1}$). In the presence of tentoxin, very large qE values were developed which relaxed more slowly. In this case, the period in the dark was extended until the rapid rise of the $F_0$ signal was completed (about 2.5–3 min).

*Inhibition by tentoxin*

When increasing concentrations of tentoxin were supplied to spinach leaf discs, there was a progressive inhibition of photosynthesis (Fig. 1A). We investigated whether this inhibition showed the characteristics expected from an inhibition of ATP synthesis. There was a progressive increase of $Q_A$ reduction and $qNP$ (Fig. 1A). The dark adapted $F_0$ value remained unchanged, and the $F_m/F_0$ ratio decreased only slightly, from 5.3 to 4.8 at the highest tentoxin concentrations used (data not shown). There was a general depletion of

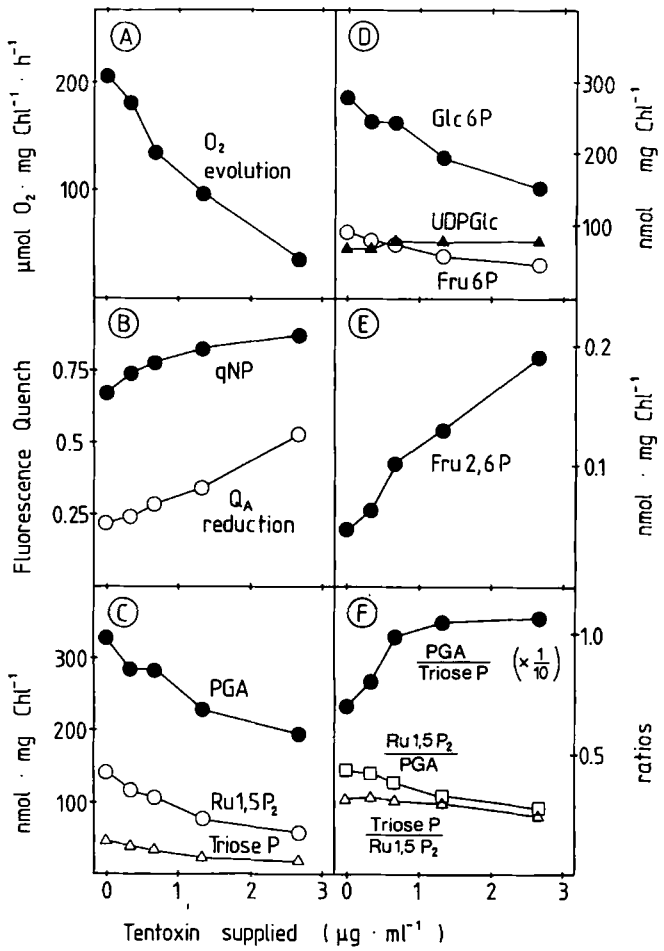


Fig. 1. Inhibition of photosynthesis by tentoxin. (A) $O_2$ evolution (●). (B) Non-photochemical quenching (●) and photochemical quenching (○). (C) PGA (●), Ru-1,5-$P_2$ (○) and triose-$P$ (△). (D) Glc-6-$P$ (●), Fru-6-$P$ (○) and UDPGlc (▲). Fru-2,6-$P_2$ (●). (F) Ratios of PGA/triose-$P$ (●), triose $P$/Ru-1,5-$P_2$ (△), and Ru-1,5-$P_2$/PGA (□). Spinach leaf discs were floated overnight on water containing tentoxin as on the figure legend, and were then illuminated (302 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in saturating $CO_2$ at 15°C for 12 min.

metabolites, including those in the Calvin cycle (Fig. 1C) and sucrose synthesis (Fig. 1D), and a 4-fold increase of Fru-2,6-$P_2$ (Fig. 1E). The PGA/triose-$P$ ratio increased progressively (Fig. 1E), while the triose-$P$/Ru-1,5-$P_2$ and Ru-1,5-$P_2$/PGA ratios remained unaltered, or fell, respectively (Fig. 1E). These results show that tentoxin is acting to restrict PGA reduction. The fluxes round the calvin cycle and to sucrose then decrease as a result of the restricted rate of PGA reduction. Tentoxin did not alter the rate of dark respiration (data not shown).

Figs. 2 and 3 investigate how tentoxin is affecting ATP synthesis and other processes in the thylakoids. The experiments were carried out at 79 (Fig. 2) and 302 (Fig. 3) $\mu mol \cdot m^{-2} \cdot s^{-1}$ illumination, allowing about 20 and 70% of light- and $CO_2$-saturated rate of photosynthesis (about 220 $\mu mol$ $O_2$ per mg Chl per h, or 31 $\mu mol$ $O_2$ per $m^2$ per s). At both light intensities, tentoxin led to a progressive and large increase of $qE$ (Figs.

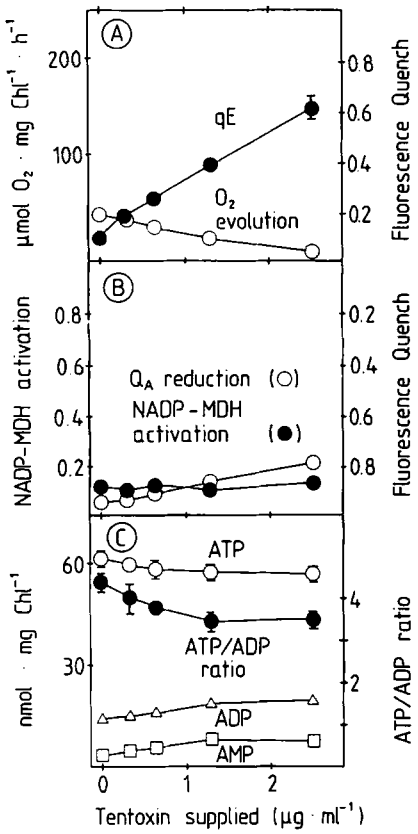


Fig. 2. Influence of tentoxin on redox states, qE and adenine nucleotides at low light. (A) $O_2$ evolution (○) and qE (●). (B) $Q_A$ reduction, estimated as (1-qQ) (○), and activation of NADP-MDH (●). (C) ATP (○), ADP (△), AMP (□) and the ATP/ADP ratio (●). Leaf discs were floated on tentoxin overnight, and then illuminated at 79 µmol·m$^{-2}$·s$^{-1}$ in saturating $CO_2$ at 15 °C for 10 min, with 700 ms flashes (3600 µmol·m$^{-2}$·s$^{-1}$) every 20 s. They were then darkened for 1 min, or until the $F_0$ value had re-established, then given a 700 ms flash (3600 µmol·m$^{-2}$·s$^{-1}$) for determination of qE. They were then illuminated for another 6 min before quenching in $N_2$ for NADP-MDH and metabolite analysis. This allowed determination of *qQ* and *qE* for each individual sample. The results are the mean ± S.E. of 4–5 replicates; the S.E. bars are omitted when they are smaller than the symbol.

2A and 3A), a decrease of ATP, and an increase of ADP and AMP (Figs. 2C and 3C). There is a statistically significant decrease of the ATP/ADP ratio.

Consumption of NADPH in the Calvin cycle will decrease when ATP synthesis is inhibited. We might therefore expect redox equivalents to accumulate in the electron-transport chain. A progressive decrease of the photochemical quench confirms that $Q_A$ does become more reduced (Figs. 2B and 3B). However, the activation of NADP-MDH remains unaltered at low light (Fig. 2B) and decreased in high light (Fig. 3B). This implies that the acceptor side of PS I becomes more oxidised when photosynthesis is inhibited by tentoxin in high light.

*Inhibition by nigericin*

For comparison, we investigated how an uncoupler influenced these parameters. An uncoupler also inhibits photosynthesis by decreasing ATP, but in this case ΔpH should decrease. In preliminary experiments we found that nigericin allowed a progressive inhibition of photosynthesis which was accompanied by a collapse of the non-photochemical quench. The ATP/ADP ratio decreased, the PGA/triose P ratio rose, there was a general depletion of metabolites, and a 3-fold increase of Fru-2,6-$P_2$ (data not shown) as expected if nigericin had uncoupled photosynthesis.

Fig. 4 compares the effect of nigericin (●——●) and tentoxin (○— — —○) on $Q_A$ reduction and NADP-MDH activation. The data are normalized to the inhibition of photosynthesis. Nigericin led to a progressive decrease of *qE*, instead of the increase found with tentoxin (Fig. 4A). $Q_A$ became far more reduced with uncoupler than with tentoxin (Fig. 4B). It is also evident that NADP-MDH activation increases after adding uncoupler (Fig. 4C), as expected if low ATP is preventing the consumption of NADPH in the calvin cycle. We also carried out a double inhibitor experiment (Table I). Nigericin was added to collapse the large ΔpH gradient obtained in the presence of tentoxin. Adding nigericin also reversed the inactivation of NADP-MDH, even though tentoxin was present.

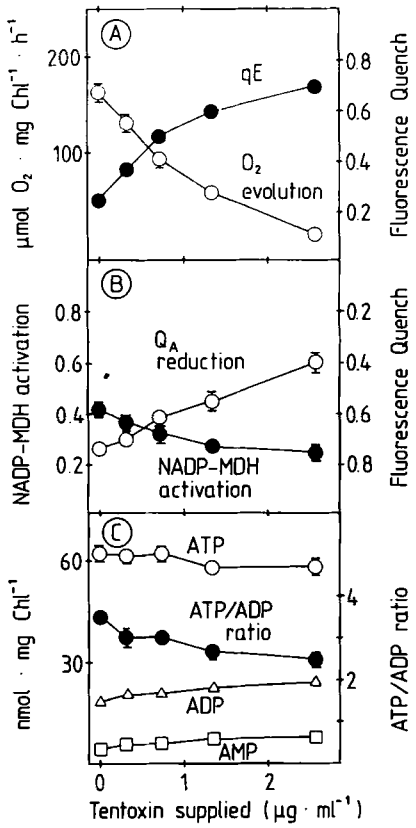


Fig. 3. Influence of tentoxin on redox state, qE, and adenine nucleotides at high light. (A) $O_2$ evolution (○) and energy quenching (●). (B) $Q_A$ reduction (○) and activation of NADP-MDH (●). (C) ATP (○), ADP (△), AMP (□) and the ATP/ADP ratio (●). The experiment was carried out as in Fig. 2 except that the light intensity was 302 µmol·m$^{-2}$·s$^{-1}$.

TABLE I

*Double inhibitor experiment with tentoxin and nigericin*

Spinach leaf discs were incubated overnight on water, containing 2.7 μg/ml tentoxin or 100 μM nigericin. $O_2$ evolution and chlorophyll fluorescence quenching were measured at 15 °C and a light intensity of 302 $\mu mol \cdot m^{-2} \cdot s^{-1}$. *qNP* refers to the total non-photochemical quench, related to the dark-adapted $F_m$ value. qE refers to that component of the non-photochemical quench which relaxed rapidly, and was estimated as in the methods. The results are the mean of four separate samples; for NADP-MDH activation the standard error is also given.

| Nigericin (μM) | Tentoxin (μg/ml) | $O_2$ evolution (μmol per mgChl per h) | $Q_A$ reduction | NADP-MDH activation | *qE* | *qNP* |
|---|---|---|---|---|---|---|
| 0 | 0 | 150 | 0.15 | 0.38 ± 0.03 | 0.38 | 0.57 |
| 100 | 0 | 26 | 0.75 | 0.45 ± 0.04 | 0.12 | 0.40 |
| 0 | 2.7 | 13 | 0.60 | 0.25 ± 0.02 | 0.77 | 0.86 |
| 100 | 2.7 | 2 | 0.90 | 0.44 ± 0.04 | 0.08 | 0.35 |

This suggests the inactivation of NADP-MDH is related to the ΔpH.

*Comparison of qE and quantum yield*

There is a striking linear relation between falling rates of electron transport, $J_e$, (for estimation see Methods) and increasing values of *qE* (Fig. 5) as photosynthesis is inhibited by rising tentoxin concentrations at low (▲——▲) or high (●——●) light. The rate of electron transport extrapolated to zero as ΔpH-dependent quenching approached its maximal value ($qE = 1$). This relation becomes even simpler if the electron transport flux ($J_e$) is divided by the incident photon flux ($I$) to yield the apparent quantum yield ($\Phi_S$). In Fig. 6A, $(1 - \Phi_S)$ is plotted against *qE*. When the quantum yield decreases, there must be an equivalent increase of energy dissipation. This plot, therefore, compares energy-dependent quenching with the probability that the energy from an incoming photon will be dissipated, rather than being used for photochemistry. There is a simple stoichiometric relation which extrapolates to a quantum yield of near zero when $qE = 1$. The apparent quantum yield of 0.11 when $qE = 0$ corresponds closely with the expected quantum yield for $CO_2$ dependent $O_2$ evolution (8–9 quanta/$O_2$, Ref. 29).

This simple relation breaks down if the total non-photochemical quench is plotted, instead of energy quenching (Fig. 6B). There is a linear relation, but it approaches the expected maximum quantum yield when 40–50% of the non-photochemical quench is still present. This can be understood because a substantial part of the non-photochemical quench in these conditions is not related to energy quenching [13,14], and some of these other components may not lead to energy dissipa-

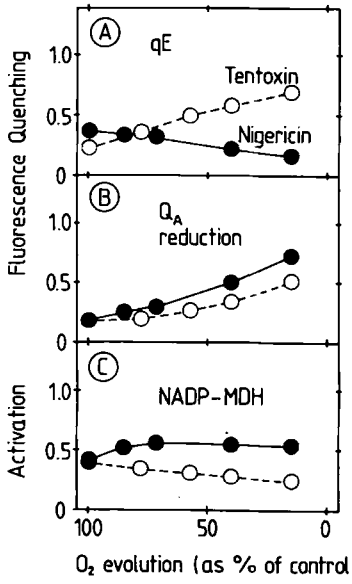


Fig. 4. Influence of nigericin on *qE*, $Q_A$ reduction and activation of NADP-MDH. (A) *qE*. (B) $Q_A$ reduction. (C) NADP-MDH activation. The experiment was carried out as in Fig. 2, except that nigericin was used instead of tentoxin, and the light intensity was 302 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The results are plotted against the % inhibition of photosynthesis; the rate in uninhibited leaves was 151 μmol $O_2$ per mg Chl per h. The result with nigericin are shown as solid symbols (●——●); for comparison, results with tentoxin are included as open symbols (○— — —○).

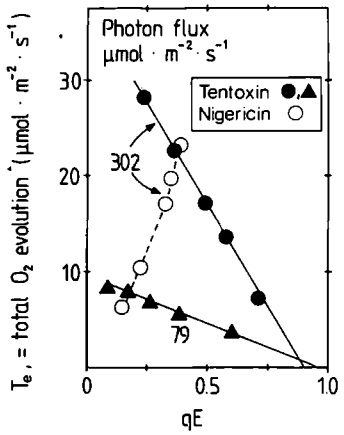


Fig. 5. Relation between the estimated rate of electron transport and *qE*. The result are for rising tentoxin concentrations at 79 (▲——▲) and 302 (●——●) $\mu mol \cdot m^{-2} \cdot s^{-1}$, and rising nigericin concentrations (○— — —○) at 302 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The rate of electron transport was estimated as the net rate of $O_2$ evolution plus the rate of dark respiration assuming photorespiration to be negligible, and is expressed as $O_2$ equivalents (see Methods).

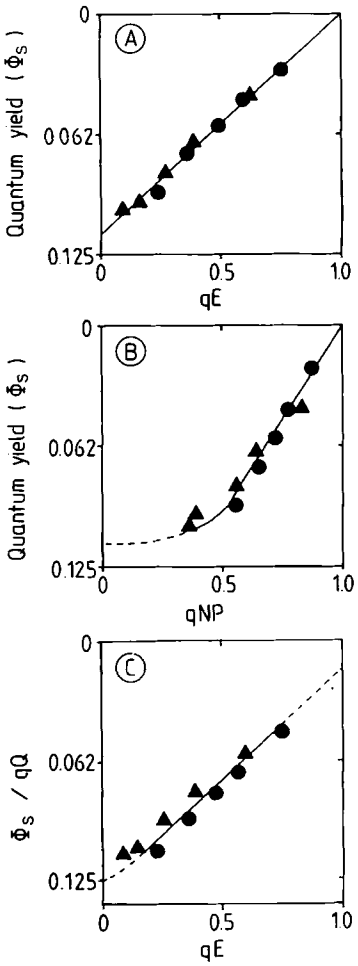


Fig. 6. Estimation of the relation between *qE*, *qQ* and the rate of energy dissipation via pH-related and redox-related mechanisms. (A) Apparent quantum yield ($\Phi_S$) vs. *qE*. (B) Apparent quantum yield vs. the non-photochemical quench. (C) Photochemical yield ($\Phi_P = \Phi_S/qQ$) vs. *qE*. The apparent quantum yield is estimated as $J_e$ per photon flux.

tion. For example, a large part is due to state transitions, especially at low light [14]. Intercepts of this kind have been seen in previous studies of energy-dependent quenching and quantum yield [12,13]. It is possible that these studies may have included part of the state transition in their estimate of energy quenching, especially in low light (see discussion in Ref. 13).

## Discussion

*Contribution of Δ pH-dependent energy dissipation*

If the light intensity is maintained constant and photosynthesis is inhibited, an increasing amount of light energy will have to be dissipated. The mechanisms involved in this energy dissipation will have to be tightly regulated, because otherwise it would not be possible to utilise low light intensities in an efficient manner. One potential mechanism is a ΔpH-dependent increase in energy dissipation [1–3,7,12,23], which is revealed by energy quenching. The correlation between apparent quantum yield and energy quenching after adding tentoxin (Fig. 6A) suggests there could be a simple stoichiometric increase in energy dissipation as *qE* increases.

The mechanisms involved in qE are not known. However, Weis and Berry [12] and Weis et al. [15] have proposed that energy quenching involves a change from a photochemically active PS II reaction centre (PS $II_0$) into a form with a low photochemical yield and high thermal energy dissipation (PS $II_e$). The PS $II_e$ centres would allow rapid energy dissipation, and minimise the extent to which energy has to be dissipated via closed $PS_0$ centres. Their analysis of the light saturation curve of sunflower suggested the photochemical yield of the postulated PS $II_0$ centres was 2–3 fold lower than the PS $II_0$ centres. Following their analysis, we have divided the apparent quantum yield ($\Phi_S$) by the fraction of open PS II traps (that is, photochemical quenching) to estimate $\Phi$p, the apparent quantum yield of the open PS II centres. The photochemical yield is plotted against qE in Fig. 6C. A near-linear relation is obtained which extrapolates to a residual value of $\Phi p = 0.015$ at $qE = 1$, implying that the postulated PS $II_e$ centres would be 8–9-fold less efficient than the PS $II_0$ centres. This is a larger change than suggested by Weis and Berry (1987). These differences may be due to technical problems in estimating *qE*. The sunflower plants were grown in very high sunlight, which makes it more difficult to obtain a fully saturating light pulse (O. Bjorkman, personal communication). Their estimate was also made using a flash after 5 min dark which may mean other components apart from qE were included (see above). However, our higher values will also only be an approximation, as small errors are unavoidable when resolving *qE* from other components of the non-photochemical yield in leaves.

*Influence of Δ pH on electron transfer to NADPH*

The redox state of the stromal NADP(H) system, as monitored by activation of NADP-MDH, become more oxidised after adding tentoxin (Fig. 3A). In contrast, $Q_A$ became more reduced. This suggests there is a restriction on electron transport between the two photosystems. Tentoxin has no known direct effect on uncoupled or basal electron transport in thylakoids [17,18]. We therefore suggest that tentoxin is inhibiting electron transport indirectly. The inhibition of electron transport must be quite strong, because $Q_A$ becomes more reduced even though energy dissipation via qE is also increasing (see above).

These results can be compared with the earlier observations that PS I becomes more oxidised when the light intensity is increased, or $CO_2$ is reduced [15,16]. A similar overreduction of PS II and overoxidation of PS I acceptors is also seen immediately after lowering the

light from super-saturating intensities [25]. In all these cases, the non-photochemical quench was high, and it was suggested that high ΔpH may contribute to the shifted relation between the two photosystems. The results with tentoxin are quite consistent with this interpretation. When the ATP synthase is partially inhibited by tentoxin, the thylakoid ΔpH must rise to a higher level to reach a given rate of ATP synthesis [30]. The higher values of *qE* which we observed after adding tentoxin are consistent with thylakoid energisation having increased. The rising ΔpH would then restrict electron transport and inhibit NADPH formation. The observation that the $Q_A$/NADP · MDH ratio starts to increase at the lowest tentoxin concentrations which we used, when qE only increased slightly, raises the possibility that the thylakoid ΔpH could already be exerting an influence on NADPH formation in the uninhibited leaves.

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