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Dissipation of excitation energy by Photosystem II particles at low pH

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The effect of decreasing the pH on the properties of Photosystem II particles isolated from pea and spinach has been investigated. Between pH 6.5 and 4.0 there was a decrease in quantum efficiency of oxygen evolution that was not associated with a proportional closure of reaction centres, as determined by the fluorescence coefficient, q_P. This decrease in the quantum yield of Photosystem II appeared to be due to two separate mechanisms. Between pH 6.5 and 5.0, the decrease in quantum yield was not associated with any change in fluorescence yield or in the ratio of F_v/F_m; the decrease was greater at limiting light than in saturating light and may be caused by a cyclic electron transfer that is associated with the destabilisation of the oxidised intermediates of the oxygen-evolving complex and an accelerated rate of Q_A^- oxidation. Below pH 5.0 the decrease in efficiency is correlated with a quenching of chlorophyll fluorescence. This quenching was light-dependent and required oxidising conditions; redox titrations indicated that a component with a midpoint oxidation reduction potential of +405 mV at pH 4.0 needed to be oxidised if the low pH-dependent quenching was to be observed. The relationship between these effects of low pH and the 'down-regulation' of Photosystem II observed under physiological conditions is discussed.

Introduction

Photosynthetic O_2 evolution has been shown to have a quantum efficiency approaching the theoretical maximum in vivo [1]. This requires a quantum efficiency of near to 1 for Photosystem II and results from a molecular organisation which promotes efficiency in energy transfer to the reaction centre, primary charge separation and stabilisation by electron transfer between secondary donors and acceptors. Although these features are physiologically important in achieving high quantum yield at limiting irradiance, it is clear that, in vivo, in a natural environment, the light intensity is often in excess of that which can be used with such maximum efficiency; indeed, these same features may be detrimental because they would promote photoinhibitory damage [2]. It has long been known that as light saturation is approached the proportion of closed PS II reaction centres increases as the quantum efficiency of photosynthesis decreases. More recently, measurements of chlorophyll fluorescence have indicated that non-photochemical thermal dissipation of excitation energy can also bring about a decrease in quantum yield of PS II [3–5]. This diversion of excitation energy means that, in excess light, PS II is actively 'down-regulated', in order to balance the light and dark reactions of photosynthesis and to provide protection from photoinhibition [6]. There is also evidence, obtained using isolated thylakoids [7–9] and algal cells

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Abbreviations: ADRY, acceleration of the deactivation reactions of Y, O_2 evolution; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; E_{m4}, midpoint potential as determined by redox titration at pH 4.0; F_o, level of chlorophyll fluorescence when PS II reaction centres are open; F_m, level of chlorophyll fluorescence when PS II reaction centres are closed; F_v, F_m minus F_o; PS II, Photosystem II; q_E, coefficient of non-photochemical quenching due to the high-energy state; q_N, non-photochemical quenching coefficient; q_P, photochemical quenching coefficient; Q_A, primary quinone acceptor of Photosystem II; Q_B, secondary quinone acceptor of Photosystem II; Q_{400}, fluorescence quencher with a midpoint potential of approx. +400 mV; Z, primary tyrosine electron donor to Photosystem II; ϕ_s, quantum efficiency of O_2 evolution; ϕ_p, potential quantum efficiency (ϕ_s divided by q_P); ϕ_{II}, quantum efficiency of Photosystem II; ΔpH, transthylakoid proton gradient.

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[10], that futile electron transfer pathways may operate under conditions of high irradiance to add to the protective effect of direct dissipation of excitation energy.

The experimental rationale that has enabled characterisation of the regulation of PS II is based on the quantitative analysis of chlorophyll fluorescence (for a recent review, see Ref. 11). Two levels of fluorescence provide the starting point for this analysis: when all PS II reaction centres are open a minimum fluorescence yield, F_o, is found, and when all centres are closed, a maximum yield F_m is obtained. The maximum quantum yield of PS II photochemistry is then defined in terms of the ratio of variable fluorescence ($F_v = F_m - F_o$) to F_m. Photosynthetic electron transport to NADP (and CO_2 reduction) will keep reaction centres open and so quench the fluorescence from the F_m level; the utilisation of excitation energy to drive photosynthetic electron transport is described by the quenching coefficient, q_P (formerly referred to as qQ), which is a measure of the proportion of open PS II reaction centres. However, photochemical quenching is not the only quenching process that occurs during photosynthesis in vivo; the increase in thermal dissipation of excitation energy causes non-photochemical quenching of fluorescence, described in a variety of ways, including q_N, the coefficient of non-photochemical quenching. Non-photochemical quenching brings about a decrease in F_v/F_m, this ratio being referred to as F_v'/F_m' in the quenched condition. It has been concluded, therefore, that the quantum yield of PS II electron transport (ϕ_{II}) in any condition can be quantified by the product of q_P and F_v'/F_m' [5]. The efficiency of PS II calculated in this way can then be compared to the directly measured efficiency of photosynthetic electron transport (ϕ_s) obtained from the rate of oxygen evolution or CO_2 uptake divided by the light intensity. It has been shown that in a range of plant species, under a variety of conditions, there is a fixed relationship between ϕ_{II} and ϕ_s which is linear except at the highest values of quantum yield [5,12]. Deviations from this relationship provide evidence for other responses of the photosynthetic apparatus to changing conditions; thus, decreases in ϕ_s which are greater than those seen in ϕ_{II} would indicate increases in dissipative photochemical processes such photorespiration, the Mehler reaction or a futile PS II cycle [8].

A total estimate of the effect of wasteful dissipative processes on photosynthetic quantum efficiency is derived by dividing ϕ_s by q_P, to give a term called the 'intrinsic yield' of PS II, ϕ_p [3]. ϕ_p is therefore the quantum yield of photosynthesis corrected for the presence of closed centres. This parameter is decreased by q_N because thermal dissipation reduces the proportion of absorbed excitation energy that is available to the reaction centres. Photochemical dissipation causes a decrease in ϕ_p because it increases the proportion of charge separations that do not give rise to measured net photosynthesis. Measurements made on a range of systems (algal cells, plant cell protoplasts, intact chloroplasts, thylakoids and leaves) have provided evidence for dissipation by both photochemical processes and q_N [3–5,7–9,13–15]. Both q_N and dissipative electron transfer are dependent on the presence of a ΔpH [9]. At present there is no identification of the mechanism by which the ΔpH can cause these changes in electron transfer and light-harvesting in PS II, although it is most likely a result of acidification of the thylakoid lumen. Indeed, acidification of thylakoid membranes has been shown to increase q_N [16] and inhibit electron donation from the O_2-evolving complex to the PS II reaction centre [17].

Therefore, a possible way to increase our understanding of the regulation of PS II by ΔpH is to examine the pH-dependency of electron transfer and fluorescence quenching in PS II particles. In this paper we describe the initial characterisation of the effects of low pH on isolated 'BBY' particles and demonstrate the presence of two separate effects on photochemical efficiency.

Materials and Methods

Photosystem II particles were prepared from either peas or spinach (as indicated in the figure legends) using a modification of the original 'BBY' preparation, as described by Chapman et al. [18]. Electron transport rate was determined using dichloro-*p*-benzoquinone (25 μM) in the presence of 3 mM potassium ferricyanide in a medium containing 5 mM $MgCl_2$, 5 mM NaCl, 10% glycerol, buffered to the appropriate pH with 20 mM Mes (pH 5.0–6.5) or 10 mM sodium citrate (pH 4.0–5.0). Actinic light was provided by a 250 W tungsten halogen lamp filtered by an Ealing 680 nm short-pass filter and a heat filter. The intensity (I) was adjusted with neutral density filters, the stated values referring to those measured at the entrance to the Hansatech O_2 electrode chamber. The quantum efficiency of electron transport, ϕ_s, was determined by dividing the measured rate of O_2 evolution by I. Chlorophyll fluorescence was measured using a Hansatech modulated fluorimeter with additional filters as described previously [8]. The experimental protocol was, firstly, to record the F_o in the presence of the low intensity (approx. 1 μmol quanta/m^2 per s) measuring beam. Determination of F_m required the use of a second halogen lamp which was shutter-controlled to deliver a saturating pulse of 1 s duration. Actinic light was given, and after 2 min the steady-state level of fluorescence was recorded (F_t) and another flash was applied to record the F_m'. Generally the F_m and F_m' values were indistinguishable and no quenching of the F_o was observed. q_P was determined from $(F_m'-F_t)/(F_m'-F_o)$ and ϕ_p by the ratio of ϕ_s/q_P. Redox titration was performed essentially as described earlier

[19], using the following reagents as redox mediators at a concentration of 10 μM: phenazine methosulphate, phenazine ethosulphate, 1,2-naphthoquinone and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine, none of which caused any decrease in the F_v/F_m ratio. Sodium dithionite and potassium ferricyanide were used to adjust the redox potential which was measured using an EIL redox electrode calibrated with acidified ferric/ferrous ammonium sulphate (E_h + 674 mV). Samples were allowed to equilibrate for between 1 and 10 min at each redox potential prior to determining the F_v/F_m ratio. Cytochrome *b*-559 was assayed using an SLM DW2000 dual-wavelength spectrophotometer as described by Horton and Croze [20].

Results

Fig. 1 shows the dependency of the quantum yield of oxygen evolution, in the presence of dichlorobenzoquinone, on pH. Below pH 6 there is a steady decline in yield, as reported previously [21]. The light intensity was chosen to be approx. 15% of the light-saturated rate of oxygen evolution at pH 6.3 in order to make it possible to examine accurately the effect of pH on q_P. It was found that the decrease in q_P as the pH was lowered was significantly less than the decline in ϕ_s (Fig. 1). In fact, between pH 6.5 and 5.0, q_P was constant at approx. 0.65, whereas ϕ_s decreased by more than 50%. Below pH 4.5 it was impossible to determine q_P because of severe fluorescence quenching (see below). The parameter ϕ_p, the intrinsic photochemical yield of PS II electron transport was found to decrease steadily below pH 6.8, at pH 4.5 declining to a value of approx. 30% of its maximum.

There are two possible explanations for the decline in ϕ_p at low pH. Firstly, there may be non-radiative dissipation of excitation energy, observed as q_N, which would decrease the energy available to drive photochemistry. Alternatively, there may be wasteful photochemistry resulting in Q_A being kept oxidised by processes that do not lead to O_2 evolution; processes that might result in this comprise a cyclic flow of electrons around PS II and electron transport to O_2.

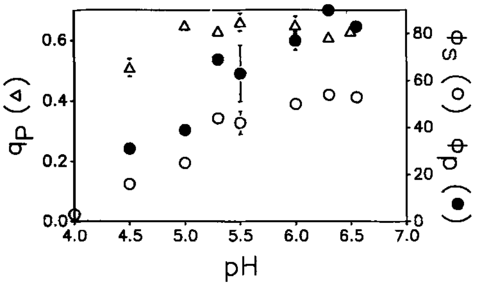

Fig. 1. Effect of pH on q_P (triangles), ϕ_s (open circles) and ϕ_p (closed circles). Light intensity was 1500 μmol/m^2 per s at the entrance to the chamber. Assays were performed on pea PS II particles in the presence of 3 mM ferricyanide and 0.25 mM dichlorobenzoquinone as described in the text. q_P was determined by the addition of a saturating pulse (30000 μmol/m^2 per s) at the end of a 2 min illumination. The linear rates of O_2 evolution were determined during the first 2 min of illumination period. ϕ values are given in arbitrary units. Each point is an average of four separate measurements. Standard deviations are shown where they are greater than the size of the data symbol.

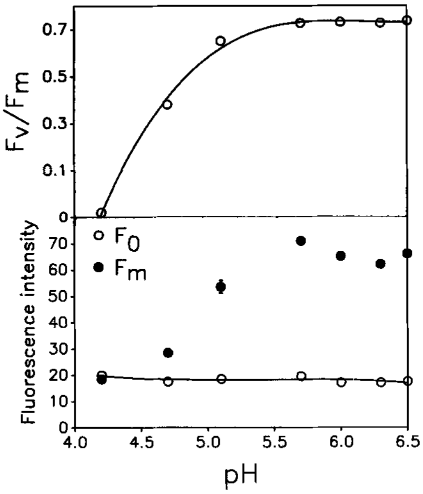

Fig. 2. Effect of pH on chlorophyll fluorescence parameters of pea PS II particles. top: F_v/F_m. bottom: open circles, F_0; closed circles, F_m. F_0 was determined using a low intensity (1 μmol/m^2 per s) measuring beam and F_m with a saturating pulse. Assays contained 3 mM ferricyanide. Each data point is an average of four assays; standard deviations were less than the dimensions of the data symbols.

In order to determine the contribution of non-photochemical dissipation to the decrease in ϕ_p, the dependency of chlorophyll fluorescence yield on pH was investigated. The F_v/F_m ratio was constant, at around 0.7, between pH 6.5 and 5.5, but fell markedly below pH 5.0. The F_0 level was largely independent of pH, whereas F_m showed a marked drop (Fig. 2). Two points were noted about the pH dependency of the decrease in F_v/F_m. Firstly, there was little change between pH 6.5 and pH 5.0, a region where there was a significant decrease in ϕ_p. Secondly, the decrease in F_m was found to be light-dependent. For instance, at pH 4.0, illumination caused the F_m to decrease below the F_0 level, hence giving rise to a negative value for the 'F_v/F_m ratio (not shown). The fluorescence quenching at low pH was further investigated and was found to be very dependent on the redox conditions. It was found that, even at pH 4.0, no quenching occurred if ferricyanide was omitted from the assay medium. The striking redox dependency of this reversible quenching is clearly illustrated in Fig. 3. Actinic light, given in the absence of oxidant, caused a rise to F_m, but addition of ferricyanide caused an immediate drop in fluorescence; this quenching could not be restored by imposition of a saturating light pulse or DCMU (data not shown). Illumination at pH 4.0 causes severe quenching, below the F_o level (Fig. 3b), but the quenching was reversed if

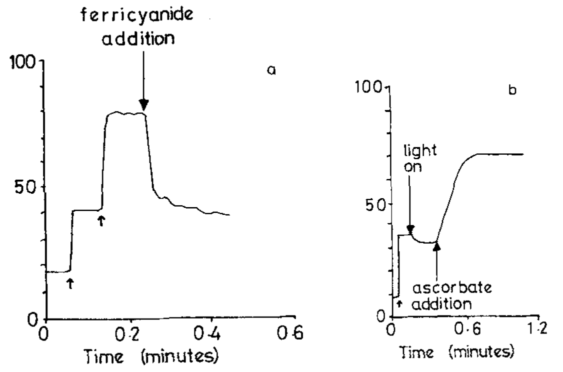

Fig. 3. Effects of redox conditions on chlorophyll fluorescence of pea PS II particles at pH 4.0. (a) Effect of 3 mM potassium ferricyanide adidtion to particles illuminated with saturating light (30000 μmol/m^2 per s); (b) effect of addition of 10 mM ascorbate to particles illuminated in the presence of 3 mM potassium ferricyanide. ↑, turning on measuring beam.

ascorbate or dithionite (not shown) was added. Clearly, oxidising conditions are required for the observation of this quenching.

A redox titration of the F_v/F_m ratio was performed to establish the nature of the oxidant (Fig. 4). At potentials below 280 mV, a decline in F_v/F_m was observed that was only associated with an increase in F_0; this is presumably a titration of Q_A/Q_A^-, which at pH 4.0 would be expected to have an E_m value approx. 180 mV more positive than at pH 7.0. Maximum F_v/F_m values, around 0.6, were recorded between 280 mV and 320 mV. At higher redox potential, the F_v/F_m declined in a way that was fitted by an $n = 1$ transition, with $E_m + 405$ mV. The decrease in F_v/F_m was reversed if the redox potential was returned to 300 mV, unless the potential had been previously taken to a value greater than 500 mV; in that case the quenching became increasingly irreversible. Thus, the fluorescence quenching at pH 6.3, shown in Fig. 4, which was observed only at very high redox potentials, was irreversible.

There are two PS II components that have been described in the literature as having midpoint oxidation-reduction potentials in this range. The first is the component Q_{400}, which has been associated with the Fe atom located in the Q_A-Q_B region of the reaction centre [22]. However, although its midpoint potential has been measured as +370 mV at pH 7.5, its reported pH-dependency would suggest a value of much greater than 400 mV at pH 4.0 [23]. The second is cytochrome *b*-559. The redox properties of cytochrome *b*-559 were therefore assessed in the PS II particles. At pH 6.3, high-potential cytochrome *b*-559 accounted for approx. 50% of the cytochrome. Ferrocyanide is an appropriate reductant for high-potential cytochrome *b*-559, since it has a pH-independent redox potential; it has previously been used as an indicator of cytochrome *b*-559 at low pH in chloroplast thylakoids [24]. Fig. 5 shows that between pH 6.5 and 4.0 there is a continuous loss of high-potential cytochrome *b*-559. The total cytochrome content, as determined by reduction with dithionite, was unchanged between pH 6.5 and 4.5, below which some loss of amplitude occurred. Therefore, at pH 4.0, where the fluorescence quencher has an E_m of +405 mV, there is no high-potential cytochrome *b*-559 detectable. It is therefore highly unlikely that quenching is associated with this cytochrome.

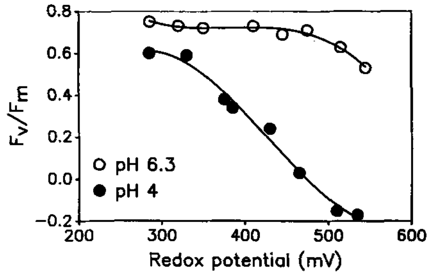

Fig. 4. Redox titration of the F_v/F_m ratio in spinach PS II particles at pH 4.0 (closed circles) and pH 6.3 (open circles). Chlorophyll fluorescence was determined as described for Fig. 2.

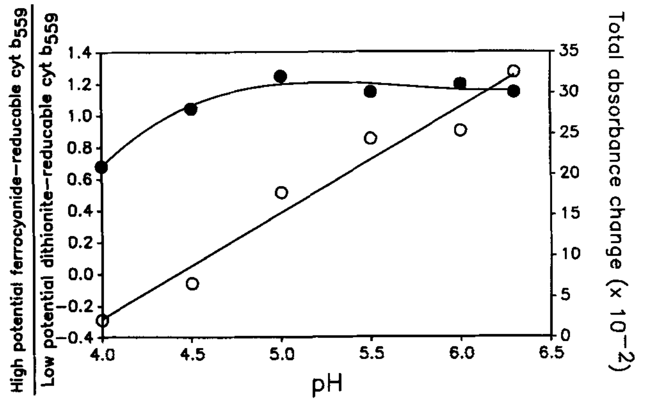

Fig. 5. Effect of pH on the content of high potential cytochrome *b*-559 in pea PS II particles. Closed circles: ratio between the absorbance change at 560–540 nm on addition of 3 mM ferrocyanide to that observed on then adding 3 mM sodium dithionite. Open circles: total amplitude of ferrocyanide plus dithionite absorbance changes. Particles were first oxidised with 0.25 mM ferricyanide. Each data point was obtained from the sum of four separate assays.

It is notable that the loss of high-potential cytochrome *b*-559 was detected throughout the pH range examined, and that the profile was similar to that observed for the decline in ϕ_p (Fig. 1). It is therefore possible that the loss of this cytochrome form, though not related to non-photochemical fluorescence, was involved in the process by which ϕ_p is decreased photochemically. It has previously been suggested that cytochrome *b*-559 is involved in a cyclic flow around PS II [25–27]. In the presence of ADRY reagents (e.g., CCCP), it is possible to observe cytochrome *b*-559 participating in a cyclic pathway; indeed, it was suggested that this effect of an ADRY reagent, observed at low irradiance, was a simulation of the effect of the low lumen pH occurring in high light [25]. Recent work has, in fact,

TABLE I

Effect of carbonyl cyanide m-chlorophenyl hydrazone (CCCP) on quantum efficiency of pea PS II particles

Assays were performed as described in Fig. 1. Actinic light intensity was 800 μmol/m^2 per s.

[CCCP] (μM)	q_P	ϕ_s	ϕ_p	F_v/F_m
0	0.72	91	13	0.69
0.25	0.68	93	14	0.70
0.50	0.62	60	10	0.71
5.00	0.62	53	9	0.70
25.00	0.56	18	3	0.67

clearly shown that ADRY reagents can elicit large increases in q_P when added to isolated thylakoids in the absence of an electron acceptor [28]. The effect of CCCP on PS II particles carrying out electron transport was therefore compared to the effect of low pH. As shown in Table I, addition of CCCP decreased the quantum efficiency of O_2 evolution but caused a smaller change in q_P. For example, 5 μM CCCP decreased ϕ_s by 42% but q_P by only 14%. Thus, there was a marked decline in ϕ_p, similar to that observed when the pH is lowered. As with the change in ϕ_p resulting from a decrease to pH 5.0, the change brought about by CCCP occurred without any alteration of the F_v/F_m ratio.

A characteristic of the ADRY effect is that the inhibition of O_2 evolution is more marked at low light intensity (or low flash frequency in the original work of Renger and co-workers [29]). Fig. 6 shows the effect of light intensity on the quantum efficiency of O_2 evolution at pH 6.3 and pH 5.0. At pH 6.3, ϕ_s and q_P both declined in parallel as the light intensity was increased, and ϕ_p was therefore constant. This is the theoretically predicted relationship between ϕ_s and q_P when the electron transport rate is altered by light intensity and when there are no changes in the proportion of wasteful photochemistry or non-photochemical dissipation. In contrast, at pH 5.0, ϕ_s was *low* in low light, and rose to a peak before declining as saturation was approached. This pattern was not shown by q_P, and therefore ϕ_p was not constant with light intensity. Instead, there was a steady increase in ϕ_p upon increasing intensity up to a saturating level. Thus, comparing pH 6.3 and 5.0, at low light, a ϕ_p decrease of 80% was observed compared to only 30% at saturating light.

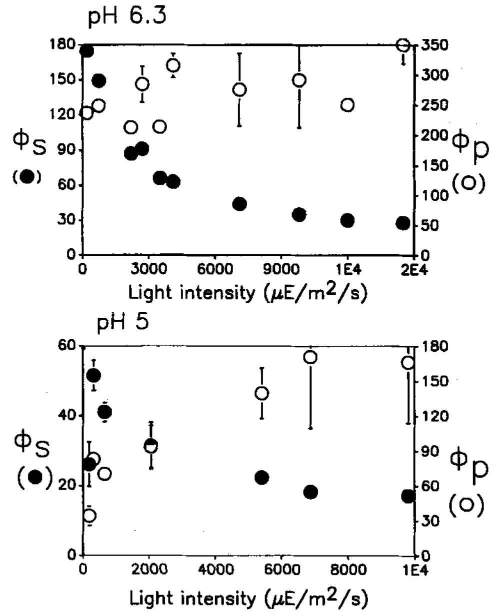

Fig. 6. Effect of light intensity on ϕ_s (closed circles) and ϕ_p (open circles) at pH 6.3 and pH 5.0. Assays were performed on pea PS II particles in the presence of ferricyanide and dichlorobenzoquinone as described for Fig. 1. Each data point is the average of four separate assays with standard deviations presented when larer than the dimensions of the data symbols.

Discussion

It is now well established that the quantum efficiency of PS II is under feedback control by the products of photosynthetic electron transport that accumulate in saturating light. It has further been suggested that this decrease in yield is associated with the build-up of the ΔpH [6]. In this paper it has been shown, to our knowledge, for the first time, that the intrinsic quantum yield of PS II can be decreased by lowering of the pH. Moreover, the change in ϕ_p resembles, in magnitude, that observed in both isolated thylakoids [7–9] and in vivo [3,4,13] in the presence of a ΔpH. The data suggest that a decrease in lumen pH is the factor responsible for feed-back control over PS II. The exact degree of lumen acidification in vivo is hard to establish. In isolated thylakoids, ΔpH values over 3.0 units have been estimated [30], suggesting a lumen pH in the range of 4.5, with an external pH of 7.5. In vivo, the stromal pH probably rises to nearly 8.0 [31] and lumen acidification may, therefore, be less than in vitro. In fact, on the basis of the observed kinetics of reduction of P_{700}, it was suggested that in vivo, at light saturation, the lumen pH does not decrease sufficiently to inhibit plastoquinol oxidation and exert classical 'photosynthetic control' [6]; in vivo, lumen pH may therefore not fall below 5.0.

The decline in ϕ_p observed here is clearly not totally dependent upon non-photochemical dissipation. Significant changes in ϕ_p were shown to occur between pH 6.3 and 5.0 without any decrease in F_v/F_m. The dependence of this phenomenon on light intensity suggests that the change in ϕ_p was caused by an effect of pH on the stabilisation of 'S' states, similar to the ADRY effect. At low light intensity the average conversion-time from S_2 to S_3 and S_3 to S_4 will increase, increasing the probability of wasteful reductive decay [29]. In the presence of an ADRY reagent the rate of this decay is enhanced [29] in a process that leads to Q_A^- oxidation.

A similar back-reaction from Q_A^- to the oxygen-evolving complex is, therefore, suggested as the explanation for the decrease in ϕ_p at low pH. This phenomenon can be observed at pH values as high as 5.0 and is distinct from the increased PS II back-reaction observed in thylakoids at pH levels less than 5.0 that caused an increase in luminescence *and* a decrease in F_m [28]. In isolated thylakoids a decline in ϕ_p has been observed which is additional to that due to non-photochemical quenching [7–9]. It was found that this photochemical component of the decrease in ϕ_p occurred at a smaller ΔpH than that needed to cause q_N, consistent with a requirement for only a modest decrease in pH shown here. Therefore, it is suggested that the effect of ΔpH on isolated thylakoids, and of pH on PS II particles, are due to the same phenomenon. However, the physiological significance of the photochemical regulation of PS II electron transfer is still to be established. In vivo, changes in ϕ_p are often small and can be completely accounted for by changes in q_N [5]. However, there are several reports of larger changes in ϕ_p [3,10,13,32], and here, photochemical dissipation within PS II could be involved. It has also been shown that changes in ϕ_p occurred without a change in q_N immediately following the onset of illumination of isolated protoplasts [33]. However, in each of these cases it has not been possible to eliminate dissipative electron transfer to O_2 as the explanation for the decrease in ϕ_p.

It is probable that the further declines in ϕ_p at pH levels towards 4.0 are related to the light-induced decrease in F_v/F_m. A number of reports exist of decreased donor-side activity at this pH; not only H_2O oxidation [17,21] but also donation by Z to P_{680}^+ is inhibited [34]. Reversible quenching of chlorophyll fluorescence at low pH has been reported previously for thylakoids [16], although this quenching may be related to gross changes in thylakoid organisation that result from neutralisation of surface negative charges and occur between pH 6.0 and 5.0 [35]. In a PS II particle it is unlikely that such changes can take place. The quenching reported here has two distinctive characteristics: firstly, it is light-dependent; secondly, it requires prior oxidation of a component with $E_{m4} = +405$ mV. The first of these makes the quenching dissimilar to energy-dependent q_N (q_E), which has been shown to quench the F_0 level in darkness [36]. Although there are measurements which indicate that q_E is enhanced under oxidising conditions in isolated thylakoids [7], there is no evidence for the tight redox dependency described here. These characteristics all suggest that the low pH-induced decrease in F_v/F_m observed in PS II particles may not relate to the in vivo q_E. However, in recent work, Demmig-Adams and coworkers [37] have suggested that q_E in vivo may consist of two components, one depending on the presence of the carotenoid zeaxanthin in the chlorophyll antenna, and the other being an effect of ΔpH on the PS II reaction centre. Although the data leading to this proposal can be interpreted by a different model [38], implicit in both models is the idea that a larger ΔpH is required for the effect on the reaction centre than that needed to cause quenching in the antenna. The particles used here do not contain zeaxanthin (Young, A., unpublished data), and it is therefore consistent with the Demmig-Adams model that low pH should induce only a reaction-centre-dependent quenching.

The identity of the +405 mV component that must be oxidised in order to observe the low pH-dependent quenching is unknown. Since the oxidation of this component does not cause any quenching prior to illumination, it is not, itself, a quencher. Instead, its oxidation may follow the formation of a light-induced quencher in the PS II reaction centre. The +405 mV component may be a donor to PS II that becomes significant only at pH 4.0, when other donors such as Z and cytochrome *b*-559 are inoperative. Alternatively, ferricyanide, itself, may be merely acting as an electron acceptor, draining electrons faster than either donation to the reaction centre or charge recombination within it, thereby promoting accumulation of the quenching species, P_{680}^+; ferro/ferricyanide has an E_m of +430 mV which is pH-independent [39].

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Note added in proof (Received 3 April 1991)

If PS II particles are prepared from zeaxanthin-containing leaves, then an additional, redox-insensitive, non-photochemical quenching is observed at low pH.

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