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THE ROLE OF pH AND MEMBRANE POTENTIAL IN THE REACTIONS OF PHOTOSYSTEM II AS MEASURED BY EFFECTS ON DELAYED FLUORESCENCE

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(1) If DCMU is added to chloroplasts which have been preilluminated (0–8 flashes) the turnover of the water-splitting enzyme is limited to one further transition upon continuous illumination. (2) The intensity of millisecond delayed fluorescence measured in the presence of mediators of cyclic electron transport around Photosystem I and of DCMU added after pre-flashing is stimulated above the level in the presence of DCMU alone and varies according to the number of pre-flashes (Bowes, J.M. and Crofts, A.R. (1978) Z. Naturforsch 33c, 271–275). (3) Separate contributions of the following energetic terms to the induction kinetics and extent of millisecond delayed fluorescence under these conditions have been examined with a view to assessing their involvement in and the mechanism of the stimulation of the emission above the level in dark-adapted chloroplasts in the presence of DCMU: (a) the initial pH of the phase in equilibrium with the water-splitting enzyme; (b) the change in internal pH which occurred when Photosystem I acted as a proton pump; (c) the electrical potential difference across the membrane resulting from rapid charging of the membrane capacitance. (4) It was confirmed that delayed light was stimulated as a result of the interaction of the intrathylakoid pH (3a and b) with the equilibria of the S-states involving proton release according to the model in which this occurs on all except the transition $S_1 \rightarrow S_2$; the stimulation was qualitatively proportional to the number of protons released. (5) There was no marked variation of the membrane potential as a function of the number of pre-flashes.

Introduction

In a previous paper [1] we presented the results of experiments in which we had measured millisecond delayed fluorescence in a phosphoroscope under conditions in which PS II was 'trapped' in particular S-states by preilluminating dark-adapted chloroplasts with varying numbers of flashes immediately before the addition of DCMU. Coupled electron transport through PS I was then initiated by the addition of reduced DAD, methyl viologen and illumination [2]. Under these conditions, the emission of delayed fluorescence varied according to the number of pre-illuminating flashes; the variation in intensity reflected the pattern of proton release accompanying transitions of the S-states which had been suggested by more direct measurements [3–6]. The results were in closest agreement with the model in which all transitions, except $S_1 \rightarrow S_2$, involved proton release according to the pattern 1, 0, 1, 2 [3,4,6].

We have now analyzed this phenomenon in greater detail and examined separately the contributions of the internal pH, transmembrane pH and electrical gradients to the stimulation of delayed fluorescence under these conditions. The results are assessed in terms of the validity of the model which relates the

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Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; DAD, 2,3,5,6,-tetramethyl-*p*-phenylenediamine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Mes, 2-(*N*-morpholino)ethanesulphonic acid; Mops, 3-(*N*-morpholino)propanesulphonic acid; PS, photosystem; Chl, chlorophyll.

observed intensity of delayed fluorescence to the interaction of the intrathylakoid pH with the equilibria of the S-states which involve proton release [1,7]. We consider factors which complicate the simple interpretation presented previously, particularly the extent to which any charge residing on the secondary acceptor B [8,9] at the time of addition of DCMU back reacts with positive charge on the donor side of PS II.

Methods

Preparation of chloroplasts

Dwarf peas (*Pisum sativum* var. Progress No. 9) were grown in vermiculite under illumination by cool white fluorescent lamps, in a cycle of 16 h light (23°C), 8 h dark (20°C). Leaves from 10–20-day-old plants were used. Chloroplasts were prepared as previously described [1], using a medium containing 0.4 M sucrose, 0.05 M phosphate buffer (pH 7.8) and 0.01 M NaCl, and stored in the dark, on ice, until use at a concentration of 2–3 mg/ml Chl. Before measurements, samples were diluted in buffer at the appropriate pH (as indicated in the figure legends) to a chlorophyll concentration of 5–15 μg/ml. Total chlorophyll was assayed according to the method of Arnon [10].

Measurement of delayed fluorescence in the millisecond range

Delayed fluorescence was measured between 1.1 and 1.6 ms with a rotating sector phosphoroscope as previously described [1,7]. Flash preillumination was provided by a xenon flash lamp (Winget, Cambridge, U.K., 200 W snaked xenon flash tube to special design; 5 μs halfwidth), connected by a fibre-optics light guide at right-angles to the photomultiplier. The phosphoroscope actinic light (12 V, 60 W, quartz halogen lamp) and xenon flash lamp were screened by blue glass filters (Corning 9782). The photomultiplier (EMI 9659, extended S-20 cathode) was screened by a Wratten 70 filter.

Unless otherwise indicated, the diluted chloroplasts were illuminated by 0–8 xenon flashes, and approx. 0.5 s after the last flash, 5 or 10 μM DCMU was added (as indicated in figure legends). Delayed fluorescence was measured on excitation by continuous actinic illumination, 15 s following this addition. Other additions are indicated in the figure legends.

Results

Interaction of Δ pH and Δψ with the S-states

An equation relating the intensity of delayed fluorescence (DF) to the concentration of the substrates, the reduced primary donor and oxidized primary acceptor of PS II, [P · Q], and to the high-energy state [11] was developed by Wraight and coworkers [7,12,13]:

$$DF = \phi_{DF} \cdot k' \cdot [P \cdot Q] \cdot \exp\left\{-\left[E^* - \left(\Delta E_{D-A\,(pH\,0)} + \left[\Delta\Psi - \frac{2.3RT}{F}\,\Delta pH\right]\right)\right]\frac{F}{RT}\right\} \quad (1)$$

where R, T and F have their usual meanings. ϕ_{DF} is a yield factor for delayed light emission, k' is a frequency factor and E^* is the singlet energy level from which emission proceeds. $\Delta E_{D-A(pH0)}$ is the redox span between the donor and acceptor pools of PS II at pH 0. The components of the high-energy state, ΔP, are $\Delta\psi$, the electrical potential difference across the membrane and ΔpH, the pH difference between the phases with which the donor and acceptor pools come into equilibrium. To avoid further complication of Eqn. 1, we have omitted equilibrium constants, which vary as a function of flash number, relating D to P and A to Q. We will refer to this point in the discussion.

If it is assumed that [P · Q], and the difference in redox poise of the components in the donor and acceptor pools (terms contained in $\Delta E_{D-A(pH0)}$) were not significantly different in the presence of valinomycin, nigericin or both, the equations given above can be simplified as follows [4], where C contains terms contributed by ϕ_{DF}, [P · Q], $\Delta E_{D-A(pH0)}$ and k' (see above):

$$DF = C \cdot \exp\left(\Delta P \cdot \frac{F}{RT}\right) = C \cdot \exp\left(\Delta\Psi \cdot \frac{F}{RT} - z \cdot 2.3\,\Delta pH\right)$$

The assumption that C = constant is of course somewhat dubious but leads to a useful analysis in terms of equations which are not prohibitively complex. With this proviso, it can be considered that the fol-

lowing ratios apply:

$$\frac{DF_{\text{no addition}}}{DF_{+\text{ nigericin}}} = \exp(-z \cdot 2.3\Delta pH);$$

$$\frac{DF_{\text{no addition}}}{DF_{+\text{ valinomycin}}} = \exp\left(\frac{F}{RT} \cdot \Delta\Psi\right)$$

where it is assumed that in the presence of nigericin, $\Delta pH = 0$ [15], so that under these conditions, $\Delta P = \Delta\psi$, and that in the presence of valinomycin, $\Delta\psi = 0$ [16], so that $\Delta P = -2.3RT/F\ \Delta pH$. If both antibiotics are present, $\Delta P = 0$. This treatment was originally suggested by Wraight et al. [14], except that a term z is now included for the number of protons involved in the reaction on the donor side.

We measured the effects of valinomycin and nigericin on the kinetics of induction of millisecond delayed fluorescence in chloroplasts preincubated in the dark for 10 min with DAD, methyl viologen and ascorbate and preilluminated with two flashes prior to the addition of DCMU (Fig. 1). The induction is biphasic under these conditions [1], and the inhibition of the relative amplitude of the fast phase by valinomycin and of the slow phase by nigericin is consistent with the assigment of a part of the fast phase

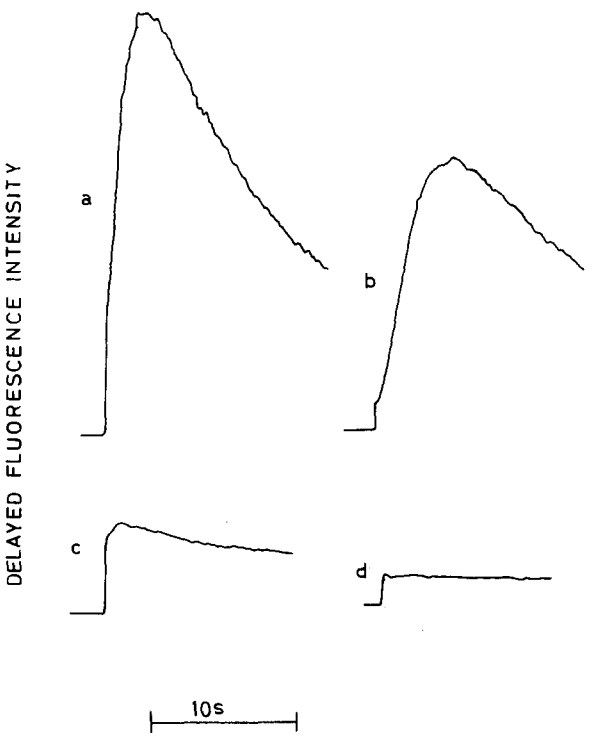

Fig. 1. Effects of uncouplers on the induction kinetics of millisecond delayed fluorescence. Pea chloroplasts were dark adapted for 10 min in 0.4 M sucrose, 50 mM phosphate buffer (pH 7.8) plus 10 mM NaCl, with 0.25 mM DAD, 1 mM ascorbate plus 0.1 mM methyl viologen, and the following: (a) no other additions, (b) 4 ng/ml valinomycin, (c) 20 ng/ml nigericin, (d) 40 ng/ml valinomycin plus 20 ng/ml nigericin. Two xenon flashes (1/500 ms) were given immediately before the addition of 5 μM DCMU. Delayed fluorescence was measured 15s later as described in Methods. Chlorophyll concentration 6.84 μg/ml.

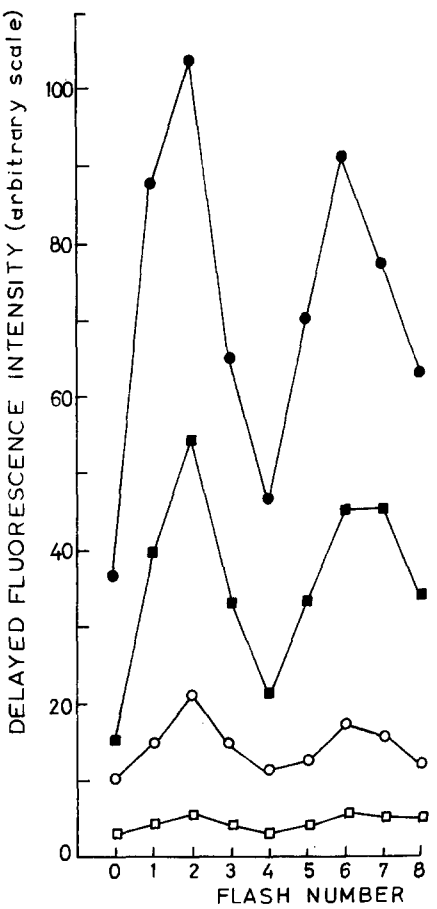

Fig. 2. Effects of uncouplers on millisecond delayed fluorescence as a function of flash number. Conditions as described in the legend to Fig. 1, for chloroplasts preilluminated by 0–8 xenon flashes prior to the addition of DCMU. •, no other additions; ▪, 40 ng/ml valinomycin; ○, 20 ng/ml nigericin; □, 40 ng/ml valinomycin plus 20 ng/ml nigericin. Maximal intensity is plotted.

(see below) to the build-up of the membrane potential ($\Delta\psi$) and the slow phase to the development of the pH gradient across the thylakoid membrane (Fig. 1). These phases are equivalent to the fast and slow phases of millisecond delayed fluorescence observed in uninhibited chloroplasts [7,17,18]. In the presence of both valinomycin and nigericin, both phases were eliminated.

Fig. 2 shows the effect of addition of valinomycin and nigericin to chloroplasts subjected to preillumination (0–8 flashes) prior to the addition of DCMU (otherwise as in Fig. 1). As observed previously [1], there is a marked oscillation in the intensity of delayed light, and the pattern is essentially maintained

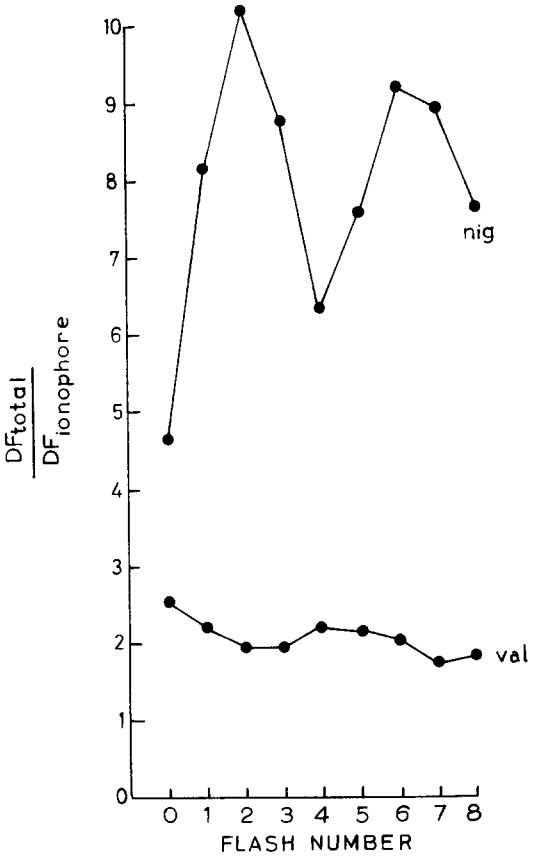

Fig. 3. Effects of uncouplers on millisecond delayed fluorescence as a function of flash number. Conditions as described in the legend to Fig. 2. DF_{total} = maximal emission in coupled chloroplasts (at 2.5 s) $DF_{ionophore}$ = emission at 2.5 s for chloroplasts with 200 ng/ml nigericin (nig) or 40 ng/ml valinomycin (val). For explanation, see text.

in the presence of valinomycin or nigericin except that it is of diminished amplitude. The oscillation was eliminated (at pH 7.8) upon addition of 400 ng/ml valinomycin plus 200 ng/ml nigericin (not shown).

ΔpH. Fig. 3 shows the portion of delayed fluorescence specifically attributable to the build-up of the pH gradient, plotted according to the simplified equations given above; a clear oscillation is evident with maxima following two or five and minima following four preilluminating flashes or in the dark sample. This is entirely consistent with our earlier proposal that the emission of delayed fluorescence is stimulated for transitions of the S-states accompanied by proton release (if this occurs according to the pattern 1, 0, 1, 2 [1,3,4,6]), by displacement of the equilibria of these transitions by the lowered internal pH: in other words, a change in the ratio $DF_{noaddition}/DF_{+nigericin}$ reflects a change in z, the number of protons released. However, the observed variations in this ratio are not explicable in terms of an integral number for z (for example, for $\Delta pH = 2$ [19], if for $z = 1$, the ratio is approx. 10, then for $z = 2$, we expect a ratio of approx. 100). We will discuss this discrepancy below.

$\Delta\psi$. Fig. 3 also shows the portion of the change in intensity attributable to the membrane potential. Jursinic et al. [20] suggested that the relative orientations of the stored charges on the S-states may be reflected in variations as a function of flash number, of the sensitivity of delayed fluorescence to reagents which dissipate the transmembrane potential. Fig. 3 shows that a variation does occur, albeit not very markedly. The amplitude of the ratio $DF_{noaddition}/DF_{+valinomycin}$ was greatest for the transitions $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$, and least for the transitions $S_3 \rightarrow S_0$ (two flashes) and $S_0 \rightarrow S_1$ (three flashes). The effect was most pronounced when the chloroplasts were preincubated at external pH 6–7, disappearing at values below 5 or above 8 (not shown). There may be several reasons why this effect is small: (i) reactions leading to separation of charge between the S-states and Q^- do not fully span the membrane and are therefore only influenced by a portion of the transmembrane field, in agreement with the anisotropic location of the water-splitting enzyme in the membrane [21,22], and with the suggestion that recombination in the millisecond range for the transition $S_1 \rightarrow S_2$ occurs across one-quarter of the mem-

brane [20]; (ii) during steady-state illumination, counter-ion fluxes balance proton uptake so that the membrane potential is low (less than 80 mV) [23]; the oscillation becomes less pronounced at lower initial values of pH_{out} (see above), presumably because the membrane potential (as indicated by the change in absorption at 515 nm) is lower at pH <6.5 [14]; (iii) according to our model in which a proton is not released in the transition $S_1 \rightarrow S_2$, we expect only S_2 to be associated with a positive charge, and since there is some evidence to suggest that this charge is counterbalanced by anions, possibly Cl^- [24], the contribution to the field of local charge differences between different states is probably minimal.

Distinction between the effect of pH_{in} and ΔpH on the S-states

In the presence of DCMU, we might consider that the actual difference in redox potential between the donors and acceptors of PS II at any particular pH consists of the following terms, assuming that the primary acceptor couple Q/Q^- is operationally pH independent [25,26] and that the primary donor is in equilibrium with one of the states of the water-splitting enzyme:

$$\Delta E' = \Delta E'_s - E'_Q$$

$$= E^o_s - z \cdot \frac{2.3RT}{F} \cdot pH_{in} + \frac{2.3RT}{F} \log \frac{[S_{n+1}]}{[S_n]} - E'_Q \quad (2)$$

where E'_s and E^o_s are the actual and standard redox potentials of the couple, respectively, and z, the number of protons involved in the one electron transition:

$$S_n \rightarrow S_{n+1} + zH^+ + e^-$$

and E'_Q is the actual redox potential of the couple $Q^- \rightarrow Q + e^-$. The pH of the internal thylakoid space with which the protons are in equilibrium is denoted pH_{in}.

Assuming that the analysis of Wraight and Crofts [7] is correct, the extent of the fast phase of delayed fluorescence observed shortly after the onset of actinic illumination can be described as follows (from Eqns. 1 and 2):

$$DF_{fast} = \phi_{DF} \cdot k' \cdot [P \cdot Q] \cdot \exp \left\{ -\left[E^* - \left(E^o_s - z \cdot \frac{2.3RT}{F} \cdot pH_{in}^{initial} + \frac{2.3RT}{F} \log \frac{[S_{n+1}]}{[S_n]} - E'_Q + \Delta\Psi \right) \right] \frac{F}{RT} \right\} \quad (3)$$

For a given redox couple, the amplitude of the fast phase can be varied experimentally by preincubation of chloroplasts at different values of pH_{out}.

During the preincubation, the pH equilibrates across the membrane; pH_{in} is the same in the presence of uncouplers. The membrane potential, developed in the first few turnovers of the photochemistry ($\Delta\psi$), depends on the functional integrity of the membrane, and can be reduced or eliminated by addition of valinomycin [15]. Two additional terms are required in Eqn. 3 to describe the total delayed fluorescence; $\Delta\Delta\psi$ describes the further change in membrane potential (a decay), which occurs as a result of redistribution of ions during the build-up of the pH gradient; and the change in internal pH, ΔpH_{in}, depends on the initial value of $pH_{in}^{initial}$ (see above), and PS I electron transport and the functional integrity of the membrane; it can be eliminated by the addition of nigericin-type ionophores, or amines of appropriate pK [27]. The effects of $pH_{in}^{initial}$, ΔpH_{in} and $\Delta\psi$ on delayed fluorescence can be experimentally isolated through the use of ionophores (see above), and by preincubation of the chloroplasts at a variety of values of external pH (pH_{out}). The results described below demonstrate these effects.

Fig. 4 shows the effect of pH on millisecond delayed fluorescence under the conditions of our experiment. We did not eliminate the small effect of membrane potential by the addition of valinomycin (Fig. 3) in these experiments. Oscillations in intensity are apparent over the range pH 5–8, consistent with the effects of pH on the reactions on the donor side of PS II [28]. The oscillations are no longer apparent at pH <5 where the water-splitting enzyme is inhibited [29]. Fig. 5 shows the maximal intensity of delayed light emitted by chloroplasts preincubated at different values of pH_{out}, and preilluminated by 25 flashes for equipartition of the S-states before the addition of DCMU. The optimum was at about pH 6.5 (unchanged by a 10-fold concentration difference of DAD, therefore not a measurement of the pK of DAD at about pH 6 [12]).

This optimum has been previously explained by a competition between two processes which contribute to the final internal pH and hence to the stimulation

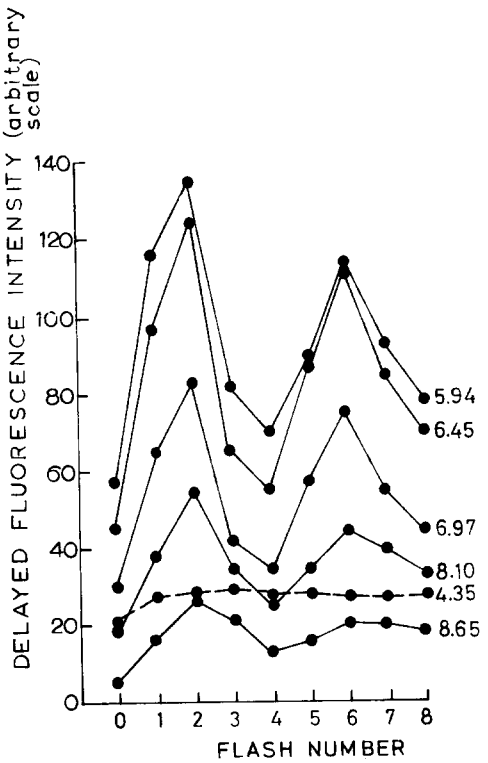

Fig. 4. Effect of pH on millisecond delayed fluorescence as a function of flash number. Conditions and protocol as described in legends to Fig. 1 and 2. Pea chloroplasts were preincubated in the dark for 10 min at the pH shown, in the following medium: 0.4 M sucrose, 10 mM NaCl plus one of the following buffers adjusted to the indicated pH; 50 mM Tricine (pH 8.65, 8.10–, 50 mM Mops (pH 6.97), 50 mM Mes (pH 6.45, 5.94), 50 mM succinate (pH 4.35). Chlorophyll concentration 5.28 μg/ml. Maximal intensity is plotted. Numbers indicate pH.

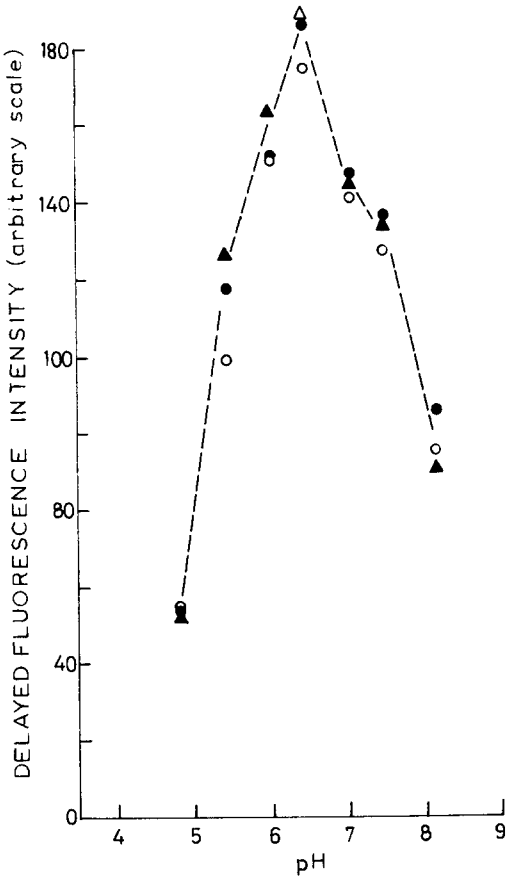

Fig. 5. Effect of pH on millisecond delayed fluorescence. Conditions as described in legend to Fig. 4, for chloroplasts preilluminated by 25 flashes prior to the addition of DCMU. Chloroplasts were preincubated with the following concentrations of DAD: ○, 0.025 mM; ●, 0.05 mM; ▲, 0.25 mM. Chlorophyll concentration 8.76 μg/ml. Maximal intensity is plotted.

of delayed fluorescence [14,19,30]: (i) proton uptake coupled to electron transport, which occurs maximally at pH_{out} ≈6 [19] and (ii) the energetic contribution of ΔpH_{in}, greatest at about pH 8.5 [19]. This is confirmed here in an experiment (Fig. 6) in which the change in internal pH (ΔpH_{in}) is inhibited. A range of amines of appropriate pK were used to eliminate ΔpH_{in} [27]. Over a limited pH range, there was no change in the overall pattern of oscillation as a function of flash number (cf. Figs. 4 and 6); under these conditions, however, the stimulation of delayed fluorescence for proton-releasing S-states is attributable to the initial value of pH_{in} (as in the case of inhibition with nigericin; see above). A marked shift in the pH optimum towards lower pH is observed when ΔpH_{in} is inhibited, as predicted by the arguments of Wraight et al. [14] (Fig. 7).

Eqn. 3 predicts that the amplitude of the fast phase of millisecond delayed fluorescence does not simply represent the portion of the emission attributable to $\Delta\psi$, but also depends on the initial pH at which the chloroplasts were preincubated. It was been previously observed that the amplitude of this phase increases at low pH values, but no explanation was offered for the effect [17]. We have isolated the fast phase into its components with valinomycin,

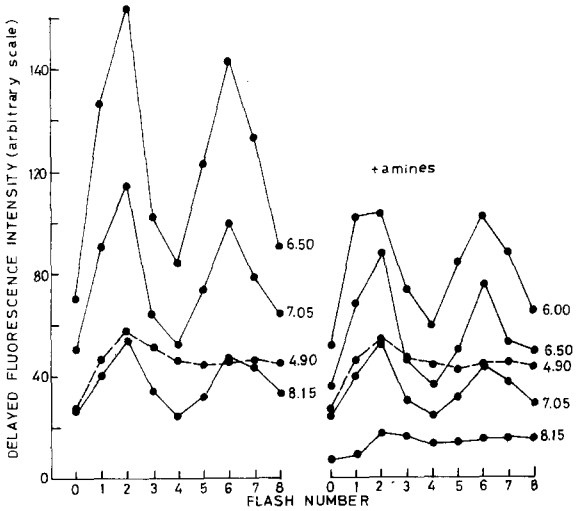

Fig. 6. Effect of amine uncoupling on millisecond delayed fluorescence as a function of flash number and pH. Protocol and conditions as described in legend to Figs. 2 and 4. The chloroplasts were preincubated without (left) or with (right) 2 mM each of NH_4Cl, pyridine and imidazole. Numbers indicate pH. Chlorophyll concentration 6.2 μg/ml. Maximal intensity is plotted.

which eliminates $\Delta\psi$ and leaves the contribution of pH_{out} ($\equiv pH_{in}$ after incubation) to this phase (Fig. 8). The amplitude of the fast phase measured under these

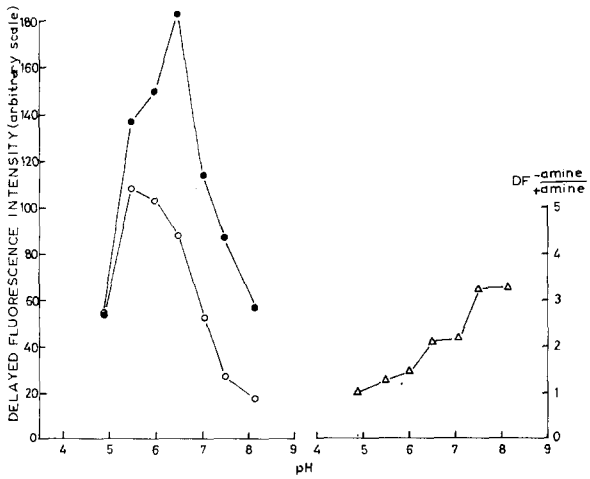

Fig. 7. Effect of amine uncoupling on millisecond delayed fluorescence as a function of pH. Conditions as described in legend to Fig. 6, for chloroplasts preilluminated by two flashes prior to the addition of DCMU. Maximal intensity is plotted. ●———●, without amines; ○———○, plus amines (see legend to Fig. 6); △———△, ratio of maximal intensities in the absence and presence of amines (for details, see text).

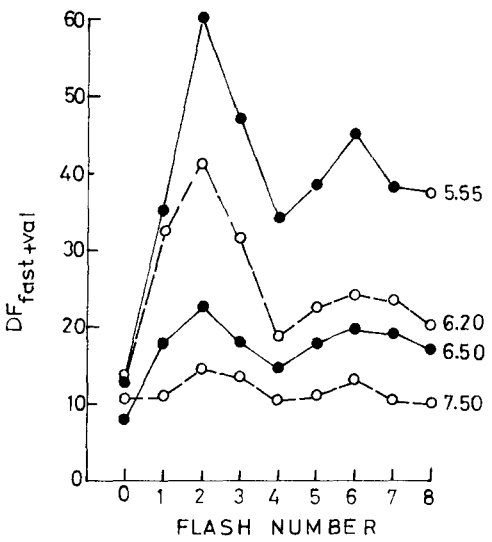

Fig. 8. Fast phase of millisecond delayed fluorescence in the presence of valinomycin as a function of flash number and pH. Protocol as described in legend to Fig. 4. Chloroplasts were preincubated in the presence of 4 nM valinomycin in the following medium: 0.4 M sucrose, 0.01 M NaCl, 0.1 M KCl plus one of the following buffers adjusted to the indicated pH: 50 mM Tricine (pH 7.5), 50 mM Mes (pH 6.5, 6.2, 5.5). Chlorophyll concentration 10.98 μg/ml. Amplitude of the fast phase is plotted.

conditions increased as the pH was lowered to a maximum of about pH 6. This amplitude showed approximately the same oscillation as a function of preilluminating flashes as that observed in fully coupled chloroplasts (Figs. 4 and 8) became damped at lower pH values (see above). This again corroborates our interpretation of the stimulation of delayed light, and supports this novel approach as a valid qualitative method for identifying the transitions of the S-states which involve proton release.

Discussion

Intensity of delayed fluorescence, internal pH and proton release

One difficulty in interpreting our results arises from the relationship between the intensity of delayed fluorescence and the stored energy driving delayed light emission, of which the internal pH is a component. Crofts and co-workers [7,12,13] considered the mechanism in terms of transition state theory, which led to the relationship shown in Eqn.

1. This relationship accounted qualitatively for the effects of ionophores and uncoupling agents on millisecond delayed fluorescence, and for the logarithmic relationship between intensity and ΔpH or $\Delta\psi$. We have previously considered (i) how this relationship might be modified to take account of the observation that different transitions of the S-states release protons to the internal phase with different stoichiometries [3–6]. We showed that, for a given transition, on changing the internal pH from one value (pH_1) to another (pH_2), the change in intensity of delayed light would be expected to follow the relationship:

$$\frac{L_2}{L_1} \propto \exp[-z \cdot 2.3 \cdot \Delta pH^{int}_{(2-1)}] \qquad (4)^*$$

In deriving this expression we assumed that the other factors from Eqn. 1 remained constant. We recognised that this was a simplification, since terms due to the changes in the redox poise of the reactants in the donor pool were ignored. A prediction which arises from this relationship is that on lowering the internal pH from the dark value to the steady-state value (driven by turnover of PS I), the extra emission of delayed light would be expected (Eqn. 4) to be an exponential function of the change in pH (the value of which is presumably independent of the S-state), and z, the number of protons released (which varies with S-state). However, this rather simple relationship is confused by the fact that for each of the transitions of the S-states, the reference redox potential of the couple involved, as well as the poise of the reaction:

$$S_n \cdot P^+ \cdot Q^- \leftrightharpoons S_{n+1} \cdot P + Q^- + zH^+$$

is different, and this will introduce a scaling factor which will differ for each transition (because Eqn. 4 refers to the ratio of the intensities of emission). We have no independent measure of the value of L_1 (the intensity of the emission at the initial value of the pH when ΔpH is zero), since a fraction of the emission seems to be rather independent of pH, electron flow, etc., and this contribution swamps L_1. Lavergne and Etienne [31] have recently reported a non-oscillating, nigericin-insensitive component of delayed fluorescence, and present arguments as to its origin which may also be applicable here. Hence, we have no way of predicting precisely the net change in intensity to be expected and the net change induced by a given change in internal pH need not necessarily vary exponentially with z from transition to transition as would otherwise be expected.

Deactivation of S-states associated with reduction of Q by B^- on addition of DCMU: Velthuys-Wollman Effect

It is now generally accepted that electron transfer on the acceptor side of PS II involves a secondary quinone, B, which accepts electrons one at a time from Q and hands them on in pairs to the plastoquinone pool [8,9]. A second difficulty with the experimental approach here is the possibility that in an important fraction of the centres, the addition of DCMU induces a rapid deactivation of the S-states because of the reduction of Q by B^- [9,32,33] and reversal of the photochemistry induced by the preceding flash:

$$S_{n+1} \cdot P \cdot Q \cdot B^- + DCMU \leftrightharpoons S_{n+1} \cdot P \cdot Q^- \cdot [B \cdot DCMU] \leftrightharpoons S_n \cdot P \cdot Q \cdot [B \cdot DCMU]$$

Several authors have discussed the extent to which such a deactivation might occur [1,31–35], and obviously, if a large fraction of centres associated with B^- became deactivated on addition of DCMU, we should take this into account in discussing our results.

In our original report [1] we concluded that DCMU-induced deactivation did not occur in a significant fraction of centres, at variance with the conclusion of Wollman [33] that a back reaction of this type occurred in a major fraction of the centres. Both Velthuys [32] and Wollman [33] commented on the fact that the amplitude of the change in F_i was less (much less in Velthuys's study) than that expected from simple models in which all centres containing QB^- were converted to Q^- [B · DCMU] upon addition of inhibitor.

The most direct experiments pertaining to this question are those of Velthuys and Amesz [35], showing that, under conditions
of preilumination similar to ours, the pattern of the S-states was not greatly affected by the addition of DCMU. We have reached a similar conclusion by

* In Ref. 1 this equation is given incorrectly without 2.3 in the exponent (DF = L).

studying the deactivation of S_2 and S_3 under the present conditions. The deactivation could be observed by a change in the pattern of oscillations when the dark time between the last preilluminating flash (either before or after the addition of DCMU) and the onset of illumination in the phosphoroscope was varied. The kinetics of deactivation of S_2 and S_3 were essentially independent of the presence or absence of DCMU (Bowes, J.M., unpublished results).

Conclusions

The major uncertainty in interpreting our results arises from the first point considered above. We can compute from established models [36] the behaviour of the S-states to be expected under our experimental conditions, and show from this the net stoichiometry of proton release expected for the transitions induced by illumination in the phosphoroscope for the various patterns proposed [3–6]. However, from the foregoing discussion, it will be apparent that we do not necessarily expect any of these patterns to correspond exactly to the pattern of oscillations observed. Nevertheless, it is clear from all our results here that the oscillations are consistent at least qualitatively with the pattern of proton release 1, 0, 1, 2, [3,4,6].

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