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THE pH DEPENDENCE OF DELAYED AND PROMPT FLUORESCENCE IN UNCOUPLED CHLOROPLASTS

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SUMMARY

1. The pH dependences of 100-ms delayed light emission and prompt fluorescence in uncoupled chloroplasts were investigated. The effects of pH on both prompt and delayed emissions could be described in a consistent manner on the basis of four phenomena distributed over a wide range of pH from pH 4 to 10.5.

2. Two effects were noted at extremes of pH (< 5.0 and > 9.5): inhibition of photochemistry and the introduction of non-photochemical quenching.

3. Stimulation of luminescence at low pH ($\leqslant 5.0$) was attributed to an inhibition of electron flow from water and a similar effect was indicated to occur at high pH ($\geqslant 8.5$). This inhibition was supported by independent measurements of the behavior of the O_2-evolving apparatus as a function of pH, using the method of flash series illumination. A buildup of the O_2 precursor state, S_3, was indicated at low and high pH.

4. An inhibition of the luminescent back reaction between the oxidizing (Z^+) and reducing (Q^-) products of Photosystem II was also noted at high pH (> 8.0). This was apparent only in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), since the increase in S_3 at high pH in the absence of DCMU opposes this inhibition.

5. The previously observed pH optimum of luminescence in coupled chloroplasts was explained in terms of the observed pH dependence of luminescence in uncoupled chloroplasts combined with the effect of the light-induced pH gradient.

INTRODUCTION

The interaction of pH with the process of delayed light production in chloroplasts has been previously studied mostly in terms of the effect of pH gradients across the thylakoid produced either by acid–base transition in the dark[1–4] or by proton uptake in the light[5]. These two approaches have led to the development of

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The terms luminescence, delayed light and delayed fluorescence are used interchangeably.

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similar models for the "triggering" of the luminescent process by the high energy state of phosphorylation[4,5].

Although the predictions of these models have, in general, been in good agreement with experimental observations one marked anomaly has been noted. The models predict that in coupled chloroplasts the nigericin-sensitive luminescence should be indicative of the stimulation of delayed emission by the pH gradient (ΔpH)[5]. This should be maximal when ΔpH is maximal and the latter has been shown to occur at external pH values of about pH 8.5[6]. However, we have previously found that the nigericin-sensitive emission (at 1 ms and 100 ms) is maximal at an external pH of about 7.0 at high actinic intensity[5,7]; for 100-ms luminescence the maximum has been shown to shift to pH 8.5 as the actinic intensity is decreased[7].

We have further investigated this problem using uncoupled chloroplasts at very low actinic intensity under which conditions the internal and external pH are the same. We conclude that the observed external pH optimum of the nigericin-sensitive luminescence in coupled chloroplasts is a compromise between a stimulation by low internal pH (at low external pH) and the stimulatory effect of a large ΔpH (at high external pH).

METHODS

Chloroplasts were prepared as described previously and total chlorophyll was assayed by the method of Arnon[9]. For emission measurements chloroplasts were suspended in 0.1 M KCl, 0.05 M buffer (adjusted with KOH/HCl) to a concentration $\times$ pathlength of 3–5 μg chlorophyll$\cdot$cm^{-2}. Nigericin ($5\cdot10^{-7}$ M) and valinomycin ($1\cdot10^{-7}$ M) were routinely added to uncouple.

Fluorescence measurements were performed in a conventional apparatus in which fluorescence was detected at right angles to the incident beam using an EMI 9558 B photomultiplier tube. Blue actinic light was obtained from a tungsten-halogen lamp by passing the beam through a water filter, a Calflex C heat filter and a Corning 4-96 filter; the intensity was varied with Balzers neutral density filters. The fluorescence emission was analyzed through a Schott RG 5 and Balzers 684 nm interference filter.

For parallel measurements of fluorescence yield and 100-ms luminescence the apparatus of Kraan *et al.*[4] was used, utilizing a single-chamber cuvette of 2 mm pathlength. Actinic light was obtained as above; the fluorescence measuring beam was passed through a combination of Corning BG 18, Corning 4-96 and Balzers B40 472 nm interference filters and was adjusted *via* the lamp voltage to give an incident intensity of 3 ergs$\cdot$cm$^{-2}\cdot$s^{-1}. The emissions were detected by EMI 9558 A photomultiplier tubes and the signal outputs were recorded on a fast, two-channel Clevite Brush chart recorder.

Measurements of the red absorption peak of chloroplasts were performed on a Cary recording spectrophotometer (Model 14 R).

O_2 evolution was measured during repetitive flash illumination[10,11] using a conventional, polarographic technique, and the a.c.-coupled signal was recorded on a Siemens Oscillomink recorder. A drop of thick chloroplast suspension was placed on a large platinum electrode, covered by a micropore filter and then bathed in buffered medium containing 1 mM NADP as electron acceptor and nigericin and valinomycin

to uncouple. No need for added ferredoxin was observed in flash experiments. Illumination was applied in the form of a series of 10 μs, saturating white flashes at intervals of about 1 s.

In order to cover a wide range of pH values four buffers were used: 2-(*N*-morpholino)ethanesulphonic acid, *N*-tris(hydroxymethyl)methylglycine, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid, and cyclohexylaminopropanesulphonic acid, all obtained from Sigma Chemical Co. All chemicals used were commercial products of highest available grade, except nigericin which was a gift from Dr R. L. Harned (Commercial Solvents Corp., Ind., U.S.A.).

RESULTS

Prompt fluorescence measurement

Fig. 1 shows the pH dependence of various prompt fluorescence parameters in uncoupled chloroplasts. The light intensity was saturating for the fluorescence rise at all pH values in the absence of electron acceptor (approx. 1.5 mW·cm^{-2} blue light). The curves are complex. Contrary to a previous report[12] the instantaneous level (F_0) was not independent of pH but showed at least four areas of pH dependence. As the pH was increased from pH 4.0, F_0 at first decreased slightly to a steady level through the neutral pH region. At alkaline pH, however, two increases occurred, one at pH 8.0 and another, steeper one at pH 9.5; at still higher values F_0 decreased.

The maximum level of fluorescence in the light (F_{max}) increased sharply as the pH was raised from 4.0 to 5.5 and then remained fairly constant until at about pH 8.5 a dramatic drop-off occurred to give a minimum at pH 9.25. Thereafter F_{max}

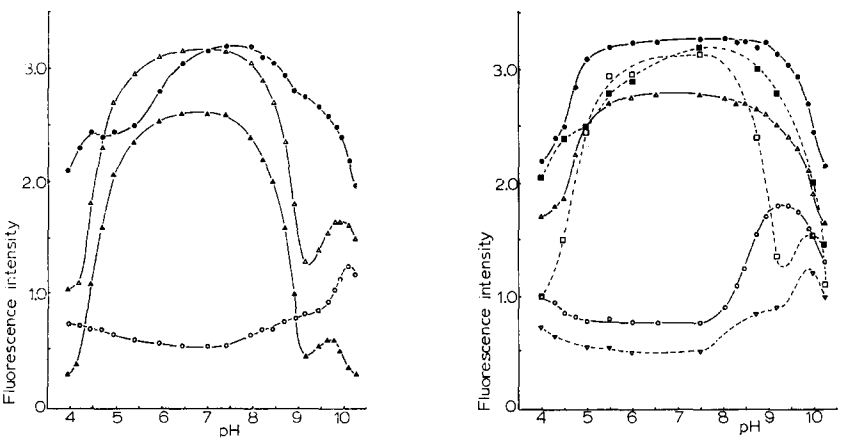

Fig. 1. pH dependence of fluorescence. Chloroplasts (5 μg chlorophyll·ml^{-1}) were suspended in 0.1 M KCl, 0.05 M buffer and preincubated in the dark for 2 min. Illumination was with blue light at an intensity of 1.5 mW·cm^{-2}. Nigericin ($5 \cdot 10^{-7}$ M) and valinomycin ($1 \cdot 10^{-7}$ M) were present initially; sodium dithionite was added to a final concentration of 0.2% after 1 min of illumination, when the light-induced fluorescence level was maximal. ○—○, the initial fluorescence level, F_0; △—△, maximum fluorescence level in the light, F_{max}; ▲—▲, light-induced fluorescence, $\Delta F = (F_{max} - F_0)$; ●—●, fluorescence level immediately after addition of dithionite, F_{dith}.

Fig. 2. pH dependence of fluorescence. Same conditions as Fig. 1 except *plus* 10 μM DCMU and light intensity was 0.45 mW·cm^{-2}. ○—○, F_0; △—△, F_{max}; ●—●, F_{dith}. Controls in the absence of DCMU are shown with dotted lines: ▽---▽, F_0; □---□, F_{max}; ■---■, F_{dith}.

roughly followed F_0. Also shown is the dithionite-reduced level (F_{dith}); this shows that Q was still present and reducible at low and high pH values where the light-induced F level decreased strongly. At the most extreme pH values F_{dith} decreased also. Addition of dithionite in the light caused an immediate rise in fluorescence which was followed by a very large, slow quenching. The quenching was light dependent and irreversible. It was also pH dependent and in the region pH 5–6 it was fast enough to more than offset the initial fluorescence increase due to reduction; since this effect was much less significant below pH 4.75, when F_{max} was greatly inhibited, or in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Fig. 2) a dependence on electron transport is indicated. No significant loss of pigment absorption was observed at 678 nm on addition of dithionite, and the loss of fluorescence is most likely due to a true quenching introduced by an interaction of dithionite (or some oxidized product) with chloroplasts in the light. At the low light intensities used for the fluorescence measurements there was no light-induced bleaching over a period of a few minutes.

In Fig. 2 the pH dependence of fluorescence in the presence of DCMU is shown for a lower actinic intensity (0.45 $mW \cdot cm^{-2}$), with a skeleton control curve in the absence of inhibitor. F_0 was raised throughout the pH range but was apparently markedly stimulated at about pH 9.0. F_{max} and the slightly higher F_{dith} both exhibited an essentially flat response from pH 5.0 to 9.0 and decreased at more extreme values. Comparison with the curve in the absence of DCMU shows that DCMU actually depressed the maximum fluorescence level in the region of pH 5 to 8.5, but raised the level at higher pH values where F_{max} in the absence of DCMU decreased anomalously.

Delosme[13] has previously reported an effect of DCMU in raising the F_0 level and has concluded that it is not a trivial effect of accidental preillumination, but the very large stimulation observed here at about pH 9.0 seemed highly suspect. In order to check this the experiments of Figs 1 and 2 were repeated at low actinic intensity (7.5 $\mu W \cdot cm^{-2}$) with great care to avoid accidental illumination. In addition, 100 μM potassium ferricyanide was added during the 2-min dark period and DCMU, when present, was added 1 min later. The results are shown in Fig. 3. It can now be seen that the large increase in F_0 at pH 9.0 in the presence of DCMU was artifactual. The general increase in F_0 over the whole pH range due to DCMU was also smaller and absent at the extremes.

ΔF in the absence of DCMU was very small, as expected for such weak actinic illumination in the presence of acceptor, and showed two small peaks at pH 8.0 and 9.5. Other experiments, not shown here, showed that at such low intensity the presence of acceptor made little difference to F_0 or ΔF in the absence of DCMU. In the presence of DCMU ΔF exhibited two stages of increase with pH, one from pH 4.5–7.0 and a steeper one from pH 7.0–9.0; at higher values ΔF decreased to zero.

Absorption changes

The red-absorption peak of chloroplasts, measured by the opal-glass technique[22], showed some pH dependence, but the behavior of the fluorescence emission cannot be accounted for by changes in the absorption. The absorbance at 678 nm decreased by about 20 % as the pH was lowered from pH 5.5 to 4.0. The absorption was roughly constant from pH 5.5–9.0, and increased by about 15 % from pH 9.5–10.5. The in-

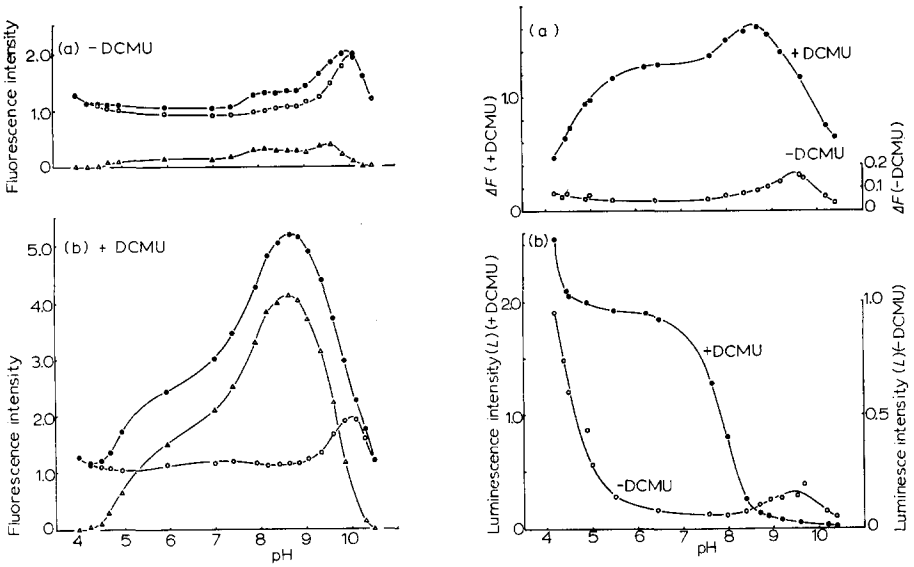

Fig. 3. pH dependence of fluorescence. Same conditions as Fig. 1 except *plus* 0.1 mM potassium ferricyanide and light intensity was 7.5 $\mu W \cdot cm^{-2}$. ○—○, F_0; ●—●, F_{max}; △—△, ΔF. (a) Fluorescence in the absence of DCMU. (b) Fluorescence in the presence of 10 μM DCMU which was added 1 min before illumination.

Fig. 4. pH dependence of fluorescence and 100-ms luminescence. Chloroplasts (10 μg chlorophyll $\cdot ml^{-1}$) were suspended in 0.1 M KCl, 0.05 M buffer. Parallel experiments, in the apparatus of Kraan *et al.*[4], determined the light-induced fluorescence (ΔF) using a very weak measuring beam (3 ergs $\cdot cm^{-2} \cdot s^{-1}$; see Methods), and the luminescence 100 ms after actinic illumination. Actinic intensity was 17 $\mu W \cdot cm^{-2}$. 10 μM DCMU was added as indicated. (a) Light-induced fluorescence, ΔF. (b) 100-ms luminescence intensity, L.

crease in absorption at 678 nm at high pH was accompanied by a 50–60 % decrease at 750 nm; no significant change at 750 nm was noted at low pH.

Luminescence and fluorescence

Luminescence and the prompt fluorescence yield were measured in parallel experiments 100 ms after very weak actinic illumination. Fig. 4 shows the pH dependence of the emissions. In the absence of DCMU the luminescence of uncoupled chloroplasts was greatest at low pH and decreased rapidly to a broad minimum at pH 7.5–8.0; it then increased again to give a small but well-defined maximum at pH 9.5. Parallel measurements of the prompt fluorescence yield showed that the light induced ΔF was very small and also exhibited a small peak at pH 9.5.

In the presence of DCMU the 100-ms delayed emission was enormously stimulated at acid and neutral pH but was depressed above pH 9.0. and showed no sign of a peak at pH 9.5. ΔF was also greatly increased by DCMU, as described for Fig. 3b. The "intrinsic" luminescence[14,15], calculated from $L/\Delta F$, is shown in Fig. 5 and it is clear that this was inhibited by DCMU. The difference between the intrinsic luminescence in the presence and absence of DCMU shows two marked maxima, at pH 4–4.5 and pH 9.0.

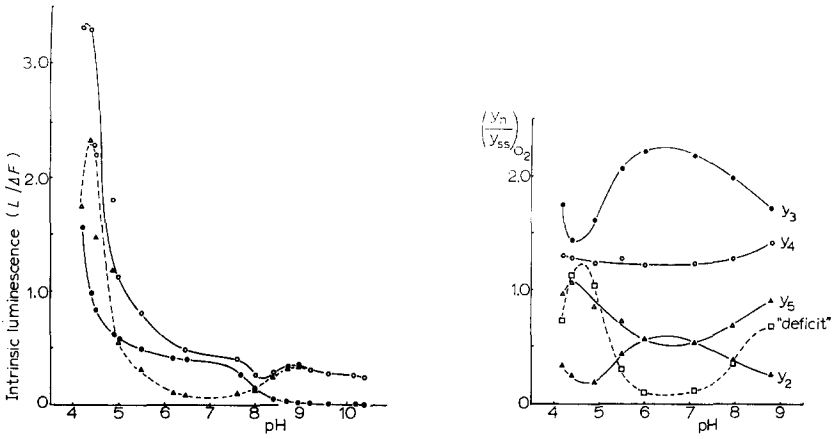

Fig. 5. pH dependence of "intrinsic" luminescence ($L/\Delta F$). From the data of Fig. 4. ○—○, intrinsic luminescence (−DCMU); ●—●, intrinsic luminescence (+DCMU); △---△, difference between (−DCMU) and (+DCMU).

Fig. 6. pH dependence of oxygen flash yields. O_2 evolution in uncoupled chloroplasts was measured during flash illumination after 3 min dark preincubation. The bathing medium was as for Fig. 1 *plus* 1 mM NADP; illumination was with a series of 25 saturating, white flashes from a Xenon lamp. Y_n is the oxygen yield of the nth flash in a series; Y_{ss} is the steady-state flash yield (25 flashes was sufficient to reach the steady state in all cases). The "deficit" is the difference between the sum of the relative yields of the first four flashes ($Y_1 = 0$ in all cases) and four-times the steady state yield.

Flash yields of O_2 evolution (Y_n)

In Fig. 6 the pH dependence of the flash yields[10,11] of O_2 production in uncoupled chloroplasts are shown, normalized to the steady state yield after 25 flashes. Y_1, the yield after the first flash, is not shown since it was negligible in all cases. It is apparent that the relative yields were strongly affected by pH. It was also found that the absolute yield of steady state O_2 evolution was strongly inhibited at extremes of pH (measurement was not possible above pH 9.0) and that the steady state was reached much more rapidly, within 5 or 6 flashes compared to 15–20 at the pH optimum (approx. pH 6.5). The strict periodicity of 4 was also lost as the pH was removed from the optimum. By comparison to the steady state yield the sum of the first four flashes shows a deficit at high and low pH which is indicative of S_4 states which did not successfully evolve O_2; Y_5 is also shown in Fig. 6 to show how S_3 states accumulate in these pH regions.

DISCUSSION

The effects of pH observed here on uncoupled chloroplasts can be interpreted as four-fold, and suffice to explain simultaneous effects on fluorescence, luminescence, and oxygen flash yields.

Considering first the fluorescence measurements: (1) At extremes of pH ($\leqslant 5.0$ and $\geqslant 9.25$) the increase in F_0 suggests that photochemistry may be inhibited. This effect is most marked at alkaline pH, possibly due to the increase in absorption described in the next paragraph.

(2) In the same extreme pH regions there is a decrease in F_{dith} and in F_{max} (+DCMU) (Fig. 2). The effects of pH on the absorption at 678 nm coincide with the fluorescence quenching, but the absorption decreases at low pH and increases at high

pH while F_{dith} decreases under both conditions. At high pH the decrease in absorption at 750 nm indicates a change in the particulate nature of the chloroplast suspension suggesting that the increase at 678 nm could be due to a decrease in the "sieve effect". The effects of pH on the absorption at extreme pH values do not alone provide a simple explanation for the decrease in F_{dith}—especially when the simultaneous increase in F_0 is also considered—and it is probable that a new quenching process is induced in addition to the absorption changes.

(3) At low internal pH electron transport is inhibited, as shown by Rottenberg *et al.*[6,16]. The data presented here suggest that this inhibition occurs near the site of electron donation by water. As the pH is raised from pH 4.0 ΔF (—DCMU) increases due to increased electron flux from water. A similar inhibition of electron flow from water above pH 8.5 causes the marked drop in ΔF (—DCMU) even at high light intensity, since electron flow from Q^- is not inhibited (Fig. 1). This effect of pH on the O_2-evolving apparatus is further supported by the data on luminescence and flash yields of O_2 production discussed below.

(4) At about pH 8.0 the back-reaction between Q^- and Z^+ is inhibited. In the presence of DCMU this is the only significant reoxidation route for Q^- (ref. 17) and its inhibition causes a further increase in ΔF(+DCMU) at very low actinic intensity (Fig. 3b). This is also responsible for the great sensitivity of the measured F_0 (+DCMU) to accidental illumination at alkaline pH. The luminescence data also reflect an inhibition of the back reaction at this pH.

No evidence for a pH dependence of the quenching properties of reduced Q was found and the dependence of fluorescence on pH observed by Kraan *et al.*[4] is most probably due to reversed electron flow to Q from secondary pools as previously suggested[7].

In addition to the effects of pH on fluorescence, Fig. 2 shows that F_{max} (+DCMU) is lower than F_{max} (—DCMU) in the range of pH 5–8.5. This effect may also be observed as a quenching of fluorescence when DCMU is added, during illumination at high intensity, to chloroplasts in the absence of electron acceptor[18]. It appears to be a quenching of fluorescence by oxidized components beyond the DCMU block, perhaps *via* Photosystem I. It is kinetically distinct from the effect noted by Delosme[13] in which DCMU increased the photochemical phase of the fluorescence rise due to decoupling of a quenching secondary electron pool, but could be mechanistically related if DCMU decoupled less effectively than reduction.

In considering the luminescence measurements it is important to ask what role the prompt fluorescence yield plays in determining the delayed emission intensity. It has been shown that for 1-ms delayed fluorescence the dependence of the delayed emission intensity on the prompt fluorescence yield is close to linear when other parameters can be supposed constant, and this has been interpreted as indicating that the prompt fluorescence yield is indicative only of the concentration of Q^- as a substrate for the luminescence reaction and is not operative as a variable yield of emission of the delayed light exciton[8]. If this first order dependence of delayed emission on the fluorescence yield is applied to the 100-ms emission measured here then, for any type of back reaction model, the "intrinsic" luminescence shown in Fig. 5 is one for which Q^- has been discounted. It is therefore a measure of the substrate, Z^+, providing the rate constant of the reaction is unchanged. This has been shown to be closely correlated to the O_2-evolving precursor state, S_3, but since a single short flash elicits some

delayed light it would seem that S_2 is also capable of producing some luminescence[19,20].

The "intrinsic" luminescence shows that extra Z^+ is produced at low and high pH in the absence of DCMU compared to in its presence (Fig. 5). The S-state "deficit" and the O_2 yield of Flash 5 (Y_5) (Fig. 6) show that the steady state level of S_3 builds up faster and is larger at high and low pH, and it is suggested that the donation of electrons from water to S_4 is inhibited causing a larger than usual proportion of S_4 to return to S_3 in a luminescent reaction resulting in a stimulation of delayed emission. The increased level of S_3 will also enhance the delayed emission and at 100 ms is perhaps the more likely cause for the observed stimulation.

Inhibition of electron flow from water at high pH ($\geqslant 8.5$) is also deemed responsible for the drop in F_{max} seen at high light intensities in the absence of DCMU. This is not seen in the presence of DCMU since only one or two electron transfers are required to reach F_{max} and no donation from water occurs until the third and fourth.

The increase in ΔF (+DCMU) at about pH 8.0 has been interpreted as indicating an inhibition of the reaction

$$Z^+Q^- \xrightarrow{k} ZQ + h\nu$$

This is also seen in the decrease in "intrinsic" luminescence at this pH. The fact that a small increase is seen at this pH in the *absence* of DCMU may be due to the increase in Z^+ more than compensating the decrease in the rate constant, k. This may be suggested by the relative sizes of the two peaks of the intrinsic luminescence compared to those of the S-state deficit. On the other hand, it may indicate a need to utilize the new model of Photosystem II proposed by Joliot and Joliot[21] in which the reaction center consists of two electron donors (Z_1, Z_2) and two electron acceptors (Q_1, Q_2), *e.g.*

$$\begin{matrix} & Z_1^+ Q_1 & & Z_1^{2+} Q_1^- & \\ (S_1) & & \xrightarrow{h\nu} & & (S_2) \\ & Z_2 Q_2 & & Z_2 Q_2 & \end{matrix}$$

In the presence of DCMU the major product of the limited turnover allowed by the blocked electron transport would be S_2, and the luminescent reversal to S_1:

$$Z_1^{2+} Q_1^- \xrightarrow{k_1} Z_1^+ Q_1 + h\nu$$

is suggested to be inhibited at high pH. In the absence of DCMU the major luminescent state is S_3 and its reversal to S_2,

$$Z_2^+ Q_2^- \xrightarrow{k_2} Z_2 Q_2 + h\nu$$

may be less inhibited.

Thus, the highly complex effects of pH on prompt and delayed fluorescence can be understood on the basis of three pH-dependent functions—photochemical turnover, the O_2-evolving apparatus and the luminescent reaction—*plus* unspecified quenching effects at the most extreme pH values (< 4.75 and > 10.0). Furthermore, the inhibition of electron flow from water at low pH and the consequent stimulation of luminescence provides a simple explanation for the anomalously low pH optimum of

luminescence in coupled chloroplasts at high actinic intensity[5,7]. Due to the light-induced proton uptake the internal pH of coupled chloroplasts in the light is considerably lower than in the external medium; thus at high light intensities the internal pH limits electron flow[16]. We have now shown that this limitation occurs at the O_2-evolving apparatus causing an accumulation of Z^+ and consequently stimulates delayed emission. When uncoupled the internal pH is higher and approaches that of the medium and Z^+ decreases due to reactivation of the water-splitting process. Thus, the observed pH optimum of the nigericin-sensitive luminescence is a compromise between the stimulatory effect of a low internal pH (achieved at low external pH) and the energetic contribution from the pH gradient (at high pH). At low actinic intensities the pH gradient is small and the internal pH is never low enough to inhibit electron donation by water and the only contributing factor is ΔpH.

The results presented here also support the suggestion that J, the rate of the luminescence reaction after "correction" for the prompt emission yield, is a measure of Z^+ and thus that the prompt emission yield is a measure only of the reduced state of Q as a substrate and does not regulate the emission of delayed light excitons[8].

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REFERENCES

1 B. C. Mayne and R. K. Clayton, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 494.
2 B. C. Mayne, *Photochem. Photobiol.*, 6 (1967) 189.
3 C. D. Miles and A. T. Jagendorf, *Arch. Biochem. Biophys.*, 129 (1969) 711.
4 G. P. B. Kraan, J. Amesz, B. R. Velthuys and R. G. Steemers, *Biochim. Biophys. Acta*, 223 (1970) 129.
5 C. A. Wraight and A. R. Crofts, *Eur. J. Biochem.*, 19 (1971) 386.
6 H. Rottenberg, T. Grunwald and M. Avron, *FEBS Lett.*, 13 (1971) 41.
7 C. A. Wraight, G. P. B. Kraan and N. M. Gerrits, *2nd Int. Congr. in Photosynth. Res., Stresa, 1971*, in the press.
8 C. A. Wraight, *Biochim. Biophys. Acta*, 283 (1972) 247.
9 D. I. Arnon, *Plant Physiol.*, 24 (1949) 1.
10 P. Joliot, G. Barbieri and R. Chabaud, *Photochem. Photobiol.*, 10 (1969) 309.
11 B. Kok, B. Forbush and M. McGloin, *Photochem. Photobiol.*, 11 (1970) 457.
12 S. W. Thorne and N. K. Boardman, *Biochim. Biophys. Acta*, 234 (1971) 113.
13 R. Delosme, *Biochim. Biophys. Acta*, 143 (1967) 108.
14 J. Lavorel, *Progress in Photosynthesis Research*, Vol. 2, H. Laupp, Tübingen, 1969, p. 883.
15 R. K. Clayton, *Biophys. J.*, 9 (1969) 60.
16 H. Rottenberg, T. Grunwald and M. Avron, *Eur. J. Biochem.*, 25 (1972) 54.
17 P. Bennoun, *Biochim. Biophys. Acta*, 216 (1970) 357.
18 C. A. Wraight and A. R. Crofts, *Eur. J. Biochem.*, 17 (1970) 319.
19 G. Barbieri, R. Delsome and P. Joliot, *Photochem. Photobiol.*, 12 (1970) 197.
20 K. L. Zankel, *Biochim. Biophys. Acta*, 245 (1971) 373.
21 P. Joliot and A. Joliot, *2nd Int. Congr. in Photosynth. Res., Stresa, 1971*, Vol. 1, p. 26.
22 K. Shibata, A. A. Benson and M. Calvin, *Biochim. Biophys. Acta*, 15 (1954) 461.