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THE EMISSION YIELD FACTOR IN DELAYED LIGHT EMISSION BY UNCOUPLED SPINACH CHLOROPLASTS

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SUMMARY

1. It is first established that the prompt fluorescence yield could, in one form or another, regulate the intensity of delayed fluorescence in two ways; as an emission yield, which would require completely random distribution of the delayed light exciton with respect to open and closed traps, and as an indicator of the reduction level of the primary acceptor, Q, if the reduced form is a substrate for the luminescence reaction.

2. The dependence of the emission yield for delayed light excitons on the overall state of Photosystem II traps has been considered on theoretical grounds and it has been concluded that the direct back reaction model for production of luminescence predicts a significantly less variable emission yield for luminescence than for prompt fluorescence.

3. Under suitable conditions a close correlation between the induction kinetics of 1-ms delayed fluorescence and the variable prompt fluorescence ($\Delta\phi^F$) is observed, which approaches a linear relationship when other variables are constant.

4. The results are interpreted on the basis that reduced Q *is* a substrate at some point in the production of luminescence and that the linear dependence of the delayed light intensity on $\Delta\phi^F$ is a reflection of this. This then implies that the emission yield for luminescence is not measurably variable and it is suggested that excitation energy transfer between Photosystem II units may not occur.

INTRODUCTION

The involvement of an emission yield factor in determining the intensity of delayed light emitted by photosynthetic systems has received increasing attention as more quantitative and detailed studies have been made. Originally proposed by Lavorel[1], the following relationships have been studied and used by many others since:

$$F = \phi I \quad (1)$$

$$L = \phi J \quad (2)$$

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-tris(hydroxymethyl)methylglycine. The terms luminescence, delayed fluorescence and delayed light are used interchangeably.

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where F and L are the intensities of prompt and delayed fluorescence, I is the actinic intensity, J is the rate of production of delayed light excitation by an unspecified mechanism and ϕ is the emission yield; the implication being that the emission yield in the two equations is the same. Using spinach chloroplasts Clayton[2] found that for delayed fluorescence in the time range of tenths of a second a reasonably quantitative correlation with the prompt fluorescence could be achieved if the prompt fluorescence yield was considered to consist of "live" and "dead" contributions. "Dead" fluorescence originates from chlorophyll *not* linked to photochemically active units, while "live" fluorescence comes from chlorophyll which is normally capable of excitation transfer to reaction centers or traps. To a good approximation the "live" contribution (ϕ_{live}) was found to be equal to the variable yield ($\Delta\phi$). Thus Eqns 1 and 2 may be replaced by:

$$F = (\phi_{\text{live}} + \phi_{\text{dead}}) \cdot I \tag{3}$$

$$L = \phi_{\text{live}} \cdot J \sim \Delta\phi \cdot J \tag{4}$$

since delayed light is generally supposed to be produced by some process occurring in active units. Clayton[2] also noted a similar behavior of 5-ms delayed fluorescence but this was largely masked by opposing changes.

Lavorel[1] has also drawn attention to the fact that if delayed fluorescence is ultimately generated by a process which opens a trap (such as the direct reversal of the primary photoact envisaged in the back reaction model):

Closed trap → open trap + exciton

then the delayed excitation should have a very high probability of recapture. Moreover, if this were the case then the overall prompt fluorescence yield is likely to be inapplicable to equations of the type 2 and 4. Fleischmann[3] has also pointed out that the use of the prompt fluorescence yield in these equations implies rapid transfer of excitation energy and communication between a large number of photosynthetic units.

Joliot and Joliot[4] and Delosme[5] have developed a model accounting for the kinetic characteristics of the prompt fluorescence which actually incorporates the concept of energy migration between units with a transfer probability (p):

$$\frac{\phi^{\text{F}}}{\phi^{\text{F}}_{\text{max}}} = \frac{(1-p)(1-q)}{1-p(1-q)} \tag{5}$$

where ϕ^{F} is the prompt fluorescence yield, $\phi^{\text{F}}_{\text{max}}$ is the maximum yield and $(1-q)$ is the proportion of closed units. By best fit with experimental data values of 0.4–0.6 were found. The derivation assumes random excitation by the incident light and a trapping efficiency in open units of 1.0 (perfect trapping); thus transfer occurs only out of closed units. It is immediately obvious that such a model combined with the back reaction model for delayed light production cannot allow any emission of delayed fluorescence excitons since they arise only in open units and are perfectly retrapped, a clear case of non-equality of emission yields for prompt and delayed fluorescence.

A more general approach to a model for ϕ^F treats the trapping efficiency in an open unit as another parameter (c) and it then becomes apparent that the prompt and delayed emission yields are not equal except as q approaches 1 (all units open). The resulting expressions of interest are:

$$\frac{\phi^F}{\phi^F_{max}} = \frac{(1-p)(1-qc)}{1-p(1-qc)} \tag{6}$$

$$\frac{\phi^L}{\phi^F_{max}} = \frac{(1-p)(1-c)}{1-p(1-qc)} \tag{7}$$

where ϕ^L is the emission yield appropriate to excitons generated only in open units and other symbols have the same meaning as before. (The derivations will be found in the Appendix.) Thus, for a model of delayed light production based on the reversal of the primary photochemistry, correction for the emission yield cannot be made simply by using ϕ^F. The extent to which ϕ^F is inadequate will depend on the values of p and c, but exact use of ϕ^F is only valid if complete random redistribution of the delayed light exciton occurs within the trapping time of an open unit in which case the modified "puddle" model[6] used for the transfer theories above is not applicable.

Other models of delayed light production which do not involve direct re-excitation to the singlet level but do utilize the reversal of the light-induced charge separation may also be considered in the light of the dependence of delayed fluorescence on ϕ^F; thus if we "allow" the exciton to escape from the influence of the open trap (for example as a triplet) then the intensity of delayed emission should exhibit a dependence on ϕ^F greater than first order, since one of the substrates for production, Q^-, is directly related to ϕ^F and the escaped exciton should be subject to ϕ^F as the emission yield. It is difficult to predict what to expect from the solid state model of Arnold[7] and Bertsch[8] since it is not well developed in detail.

It is therefore of interest to try to determine whether the observed dependence of delayed fluorescence on ϕ^F is first or second order, and it will be shown that a first-order dependence is observable under conditions when other parameters are constant, indicating that ϕ^F may not be applicable as an emission yield but only as a measure of the reduction level of Q^-.

In order to eliminate any complications due to effects of the high energy state of phosphorylation on prompt[9] or delayed[10] fluorescence all preparations were uncoupled with nigericin and valinomycin.

METHODS

Chloroplasts were prepared from fresh spinach either obtained from the market or grown in the greenhouse. Deribbed leaves were ground in a modified pestle and mortar developed by Kraayenhof[11], having grooves to allow the escape of the extruded chloroplasts from further grinding action. The grinding medium was 0.4 M sucrose, 0.05 M *N*-tris(hydroxymethyl)methylglycine (Tricine) buffer, 5 mM $MgCl_2$ 2 mM sodium ascorbate and 1 % bovine serum albumin adjusted to pH 7.6 with NaOH or HCl. After a 3-min spin at 3000 × g the pellet was resuspended in the same medium diluted 10-fold and respun for a further 3 min. The resulting loose

pellet was resuspended to a concentration of 2 mg chlorophyll per ml in a minimum volume of 0.4 M sucrose, 0.05 M Tricine, 5 mM $MgCl_2$ (or 0.01 M NaCl) and 1% bovine serum albumin adjusted with NaOH/HCl to pH 7.6. Total chlorophyll was assayed by the method of Arnon[1], measuring absorbance at 652 nm.

Delayed fluorescence was measured in a conventional rotating sector phosphoroscope with a chopping period of 2 ms. Delayed emission was monitored 0.7 ms from the end of the actinic flash (approximately 1 ms from the center). Prompt fluorenscece was measured simultaneously during the period of actinic illumination *via* a light guide monitoring front face fluorescence. Both delayed and prompt emissions were detected with EMI 9558 photomultiplier tubes and the signals passed through gated amplifiers of the type developed and described previously[10]. The time constant of the amplifier system has been improved to less than 1 ms and the limit of resolution was thus the chopping rate of the phosphoroscope. Signal outputs were recorded on a Siemens Oscillomink chart recorder using chart speeds up to 0.5 $m \cdot s^{-1}$.

The actinic light was passed through a Corning 4-96 blue filter plus a Calflex C heat filter. The maximum incident intensity was 5.5 $mW \cdot cm^{-2}$ which was found, by light titration of uncoupled electron transport, to be equivalent to about 100 s^{-1} absorbed intensity. The incident intensity was attenuated by means of Balzers neutral density filters. Delayed emission was passed through a combination of an RG 5 and RG 8 (Schott) filters and prompt fluorescence was measured through a combination of RG 2 and RG 5 (Schott) filters *plus* a 684 nm Balzers interference filter.

The cuvette was of perspex with 1-mm path length and an area of approximately 5 cm^2; chloroplasts were suspended in assay media of 0.1 M KCl, 0.05 M *N*-tris-(hydroxymethyl)methyl-2-aminoethanosulphonic acid buffer (adjusted to pH 7.5 with KOH/HCl) to a concentration of 20 μg chlorophyll per ml. Nigericin ($5 \cdot 10^{-7}$ M) and valinomycin ($1 \cdot 10^{-7}$ M) were routinely added to uncouple.

All reagents were commercial products of the highest obtainable grade. Nigericin was a gift from Dr R. L. Harned (Commercial Solvents Corp., Ind., U.S.A.).

RESULTS AND DISCUSSION

Fig. 1a shows that in the absence of any additions (except valinomycin and nigericin to uncouple) a strong similarity between the rise kinetics of prompt and delayed fluorescence of spinach chloroplasts became apparent at low actinic light intensities as the kinetics came into the time resolution of the apparatus. The divergence at longer times, due to the decline in delayed fluorescence, also decreased and was absent at the lowest intensity shown (0.25 $mW \cdot cm^{-2}$, blue light). A similar correspondence was seen in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Fig. 1b), where the subsequent decline of the delayed emission was more marked and occurred even at the lowest intensity used (0.023 $mW \cdot cm^{-2}$, and in the presence of methyl viologen (Fig. 1c) where the decline in delayed fluorescence was absent except at very high intensity. The association of this decrease with limitation of the steady-state electron flow rate strongly indicated that it was due to loss of a delayed light precursor on the oxidizing side of Photosystem II, as previously suggested by Clayton[2]. The recent results of Itoh *et al.*[13] on the effect of electron donors to Photosystem II and of inhibition of donation by water by Tris washing also supports

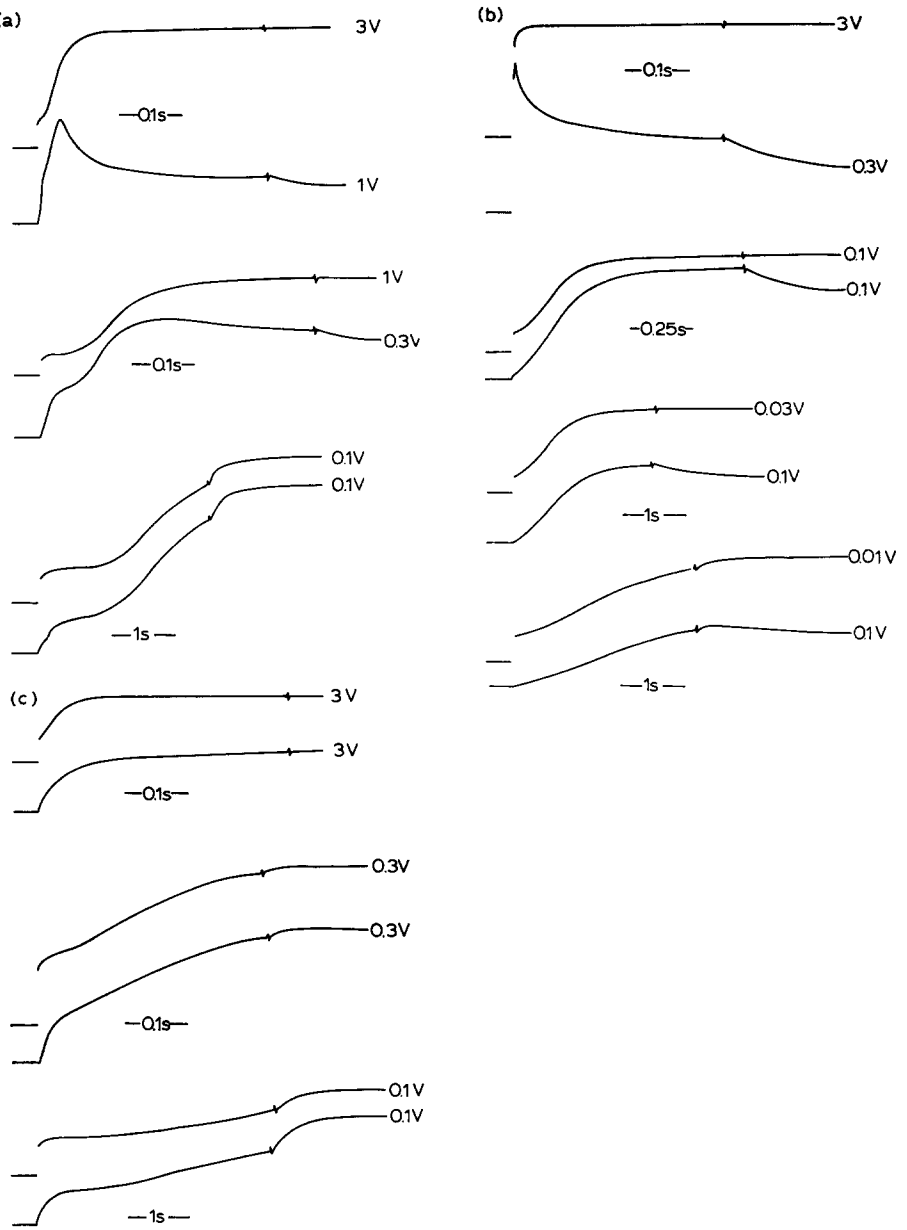

Fig. 1. Induction kinetics of 1-ms delayed light emission and prompt fluorescence in uncoupled chloroplasts. Chloroplasts (20 μg chlorophyll/ml) suspended in 0.1 M KCl, 0.05 M *N*-tris(hydroxymethyl)methyl-2-aminoethanosulphonic acid (pH 7.5), were illuminated with blue light at different intensities (100% light intensity = 5.5 mW·cm^{-2}). The upper trace of each pair is prompt fluorescence and the lower one is delayed light. The time markers refer to the initial chart speed: at the discontinuity the chart speed was reduced to 10 s per time marker in all cases. Full scale sensitivities are given in volts (V) beside each trace. (a) no additions; light intensity (numbering from the top of each group): (i) 100%, (ii) 29%, (iii) 5.3%; (b) *plus* 10 μM DCMU; light intensity: (i) 100%, (ii) 3.3%, (iii) 1%, (iv) 0.42%; (c) *plus* 25 μM methyl viologen; light intensity: (i) 100%, (ii) 29%, (iii) 5.3%.

this. This precursor is presumably related to the S-state system of the O_2-evolving apparatus which is known to undergo induction changes[14,15]. It will generally be referred to as Z^+.

In Fig. 2 the induction kinetics of delayed fluorescence are plotted against the total variable prompt fluorescence yield ($\Delta\phi_T^F$) normalized to the steady-state level which in the absence of added electron acceptor was maximal at all actinic levels used, irrespective of the presence of DCMU. At low actinic intensities, in the absence of methyl viologen, the decline to the steady-state level was less significant and it can be seen that a linear relationship between the intensity of delayed fluorescence and the prompt fluorescence yield becomes apparent, beyond an initial induction involving only about 10–15 % of $\Delta\phi_T^F$. This portion is identifiable with the initial rise in fluorescence shown by Joliot[16] and by Forbush and Kok[17] to be related to the activation reactions of the O_2-evolving system and a direct involvement of Z^+ is therefore indicated in the production of delayed light. This portion of the variable prompt fluorescence yield will be termed $\Delta\phi_Z^F$. Thus, for the major part of the prompt fluorescence rise which indicates the reduction level of Q ($\Delta\phi_Q^F$), a linear relationship holds for the delayed emission intensity.

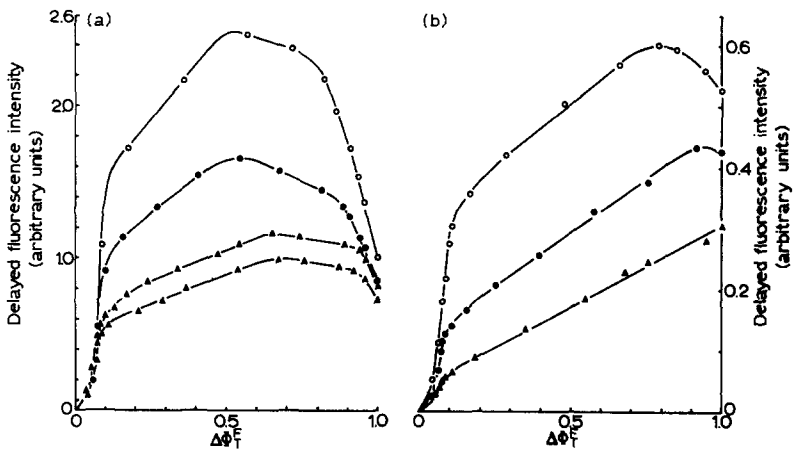

Fig. 2. Delayed fluorescence intensity as a function of the total, variable prompt fluorescence yield ($\Delta\phi_T^F$). Taken from recordings of the type shown in Fig. 1; conditions as in Fig. 1. No additions. Light intensities: (a) 100% (○); 72% (●); 41% (△); 36% (▲); (b) on expanded scale: 17% (○); 9.7% (●); 5.3% (△).

For comparison with the curves of Fig. 2 at high actinic intensities, Fig. 3 shows the induction of O_2 evolution in *Chlorella* cells as a function of $\Delta\phi_T^F$. (The figure is taken from the data of Joliot[18]; the incident intensity was specified only as "high"-probably about 10000 ergs·cm^{-2}·s^{-1}.) Since this figure was obtained for whole cells rather than uncoupled chloroplasts its use is limited to qualitative comparison but the similarity between this representation of the concentration of the state S_3 (being proportional to the rate of O_2 evolution[14]) and the curves of Fig. 2 is striking. It seems that when dark reactions become strongly limiting, as in whole cells at high intensity or in chloroplasts in the absence of added electron acceptor, S_3 does not decrease to a steady level in a simple manner. The two phases of the

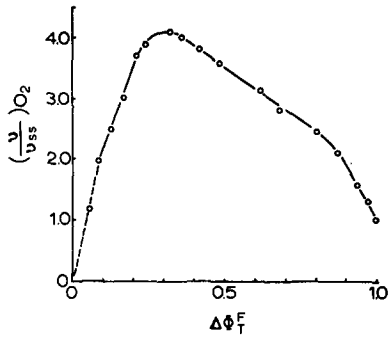

Fig. 3. Rate of oxygen evolution *vs* $\Delta\phi_T^F$. Taken from the data of Joliot[18]. Rate of oxygen evolution (v) normalized with respect to the steady state rate (v_{ss}). *Chlorella* cells: high light intensity (approx. 10^4 ergs·cm^{-2}·s^{-1}, blue light).

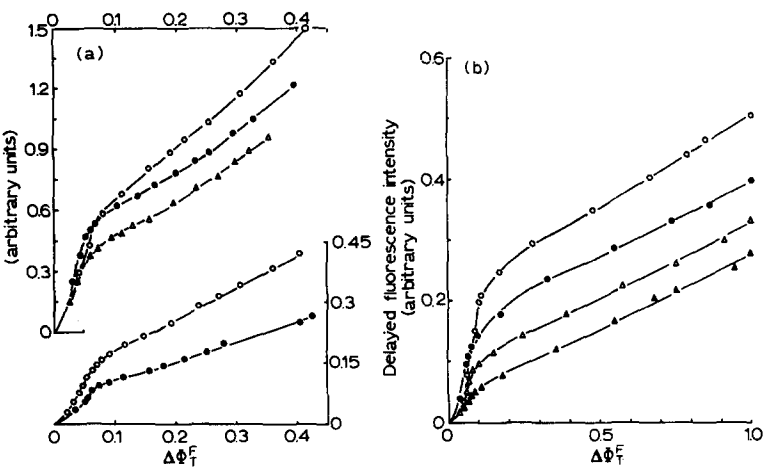

Fig. 4. Delayed fluorescence intensity *vs* $\Delta\phi_T^F$. Conditions as for Fig. 1. (a) *plus* 25 μM methyl viologen; light intensities: upper curves: 41% (○); 36% (●); 29% (△); lower curves: 9.7% (○); 5.3% (●). Same delayed fluorescence intensity scale as Fig. 2. (b) No additions, after subtraction of decline in delayed fluorescence intensity; light intensities: 17% (○); 13% (●); 9.7% (△); 5.3% (▲). Same delayed fluorescence intensity scale as Fig. 2.

decline may be related to pool sizes between the two photoreactions. Thus, the rise kinetics of delayed fluorescence cannot be readily obtained by extrapolation of the declining part except at low actinic intensities when the decrease is small anyway (Fig. 4b).

In the presence of methyl viologen as electron transport cofactor the fluorescence increase is smaller but the eventual linearity between prompt and delayed emissions is more apparent since the final rate of electron transport is not so limiting and Z^+ does not fluctuate so wildly before attaining a steady level (Fig. 4a). Thus under conditions when Z^+ is a constant but Q^- is still increasing rapidly the intensity of delayed light emission is proportional to $\Delta\phi_T^F$. On the basis of the back reaction model we might write for the intensity of delayed light emission:

$$L = k \cdot \phi^L \cdot [Z^+ Q^-] \tag{8}$$

where k is a rate constant and $[Z^+Q^-]$ is the concentration of traps in the precursor state. If Z^+ and Q^- could vary independently then the concentration of the trap species Z^+Q^- would be a product function of the individual concentrations of Z^+ and Q^-. This is not the case in general but will approach it at low light intensities as the coupling between Z^+ and Q^-, due to their simultaneous generation, is overshadowed by the independent electron transport reactions. Thus we may expect to approach the relationship:

$$L = k \cdot \phi^L \cdot Z^+ \cdot Q^- = k \cdot \phi^L \cdot Z^+ \cdot \Delta\phi_Q^F \quad (9)$$

$$= k \cdot \phi^L \cdot Z^+ \cdot (\Delta\phi_T^F - \Delta\phi_Z^F) \quad (10)$$

indicating that $\Delta\phi_Q^F$ is directly proportional to Q^- and that when Z^+ has reached a steady level L becomes proportional to $\Delta\phi_T^F$.

The singular dependence of the luminescence intensity on $\Delta\phi_T^F$ can be observed in the presence or absence of electron acceptor because at suitable actinic intensities the fluctuations in Z^+ are over fast enough – relative to the total fluorescence rise – for this variable not to interfere too strongly. This is demonstrated in Fig. 4b for low actinic intensities in the absence of acceptor when the declining phase of the delayed fluorescence induction was slow and distinct enough to be subtracted from the rise kinetics. The resulting curves show the same general shape as those in the presence of methyl viologen but the linear portion can be seen to extend over the larger $\Delta\phi_T^F$ range observed in the absence of acceptor.

In the presence of DCMU, however, Z^+ and Q^- are produced and preserved together, since electron flow from Q^- is blocked, and thus Z^+ is varying simultaneously with Q^- at all times. Under such conditions it may be expected that no linearity between delayed fluorescence and $\Delta\phi_T^F$ would be observed and this is the case (Fig. 5a). The actinic intensities employed here were much lower than for the previous figures but a decline in the delayed fluorescence was observed in all cases. At very low intensity the decline was small and slow and could be subtracted from the induction curve but the rise curves so obtained still did not exhibit a simple depen-

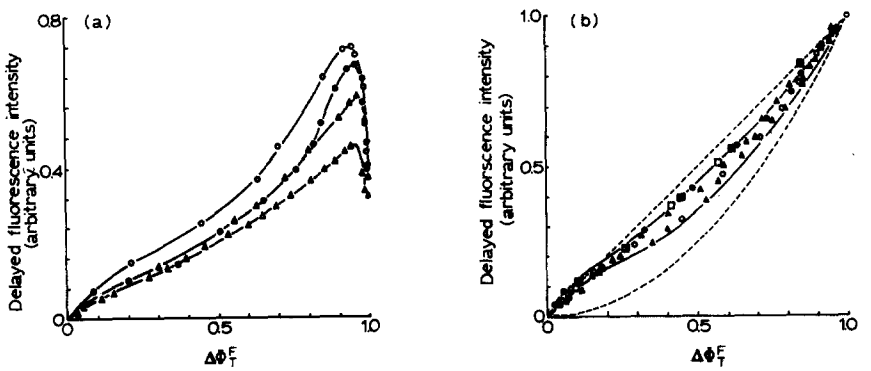

Fig. 5. Delayed fluorescence intensity *vs* $\Delta\phi_T^F$. Conditions as for Fig. 1, *plus* 10 μM DCMU. (a) Light intensities: 17% (○); 13% (●); 9.7% (△); 5.3% (▲). Same delayed fluorescence intensity scale as for Fig. 2. (b) After subtraction of declining phase of delayed fluorescence intensity; light intensities as for (a) *plus* 0.42% (□, ■). (Dotted curves show theoretical linear and square dependences.) Delayed fluorescence intensity normalized to 1.0 at maximum.

dence on $\Delta\phi_T^F$ because of the simultaneous variation of Z^+ and Q^- (Fig.5b). Indeed, it deviates in the direction of a higher order dependence, which might be expected since $\Delta\phi_T^F$ is composed of both $\Delta\phi_Z^F$ (indicating Z^+) and $\Delta\phi_Q^F$ (indicating Q^-).

The initial dependence of delayed fluorescence on $\Delta\phi_T^F$ in the presence and absence of methyl viologen (concave upwards) also deserves some comment although the data available at present do not allow a definite answer as yet. Delosme[19] has found that the initial rise in prompt fluorescence, identified with the activation reactions of the O_2-evolving system[16,17] is determined by the sum of the two S states, $S_2 + S_3$. Luminescence has been shown to be strongly (but not exclusively) related to the concentration of S_3[20,21], thus a reaction of the type

$$S_3 \cdot Q^- \rightarrow S_2 \cdot Q + \text{luminescence}$$

is indicated. Such a reaction would produce an exciton in an "open" unit (low $\Delta\phi_Q^F$) but one with a high value of $\Delta\phi_Z^F$, since S_3 is replaced by S_2. Some double dependence of the luminescence intensity on the initial fluorescence rise ($\Delta\phi_T^F \sim \Delta\phi_Z^F$ initially) might therefore be expected since this is indicative of both the concentration of Z^+ and is an open unit emission yield. The initial portion of the delayed fluorescence *vs* $\Delta\phi_T^F$ curves do indeed approximate to a square dependence (Fig. 6) but the possibility that the precursor concentration itself varies with $\Delta\phi_T^F$ in the observed manner cannot be ruled out.

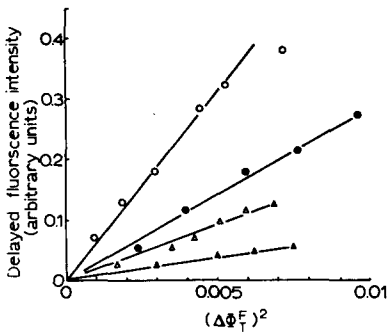

Fig. 6. Initial induction of delayed fluorescence as a function of $(\Delta\phi_T^F)^2$. Conditions as for Fig. 1; no additions. Light intensities: 29% (○); 17% (●); 9.7% (△); 5.3% (▲). Same delayed fluorescence intensity scale as Fig. 2.

Recently, Mauzerall[22] has shown that the rise in the prompt fluorescence yield is not fast enough to be associated with the primary photoact, but, although this challenges almost all the basic assumptions and concepts we have on the primary photochemistry of Photosystem II, it does not alter the fact that ϕ^F is experimentally related to Q^- and Z^+ concentrations and is an emission yield factor effective in prompt fluorescence. Thus the interpretation of the data presented here is not qualitatively affected.

FINAL DISCUSSION AND CONCLUSIONS

The observation that under appropriate conditions a linear dependence of delayed fluorescence on ϕ^F is observed has been inter preted as indicating that the

emission yield for delayed light is not the same as that for prompt fluorescence and, in fact, is not variable. The alternative possibility that luminescence is independent of the concentration of Q^- as a substrate (but proportional to the prompt fluorescence yield governed by Q^-) has not been considered because most proposed mechanisms suppose the contrary. Invariance of the delayed fluorescence emission yield (ϕ^L), or a low degree of variability, is to be expected if the mechanism of production is by direct reversal of the primary photoact resulting in singlet excitation of chlorophyll. Other models which involve Z^+ and Q^- as precursors but allow escape of the excitation from the generative unit, such as triplet or charge migration, will generally predict a variable emission yield approaching that for prompt fluorescence as well as requiring Q^- as a substrate and thus a higher than first-order dependence of delayed light intensity on the prompt fluorescence yield is expected. The predictions of the solid-state theory are uncertain; they may include a certain amount of independence from Q^-, but antiparallel behavior of delayed emission and Q^- is also expected.

The lack of dependence of the delayed emission yield on the overall concentration of Q^- implies that delayed light excitons do *not* communicate with neighboring units, either because the trapping rate is very high (c in Eqns 6 and 7 is near 1) or because excitation transfer between units is very limited (p in Eqns 6 and 7 is near 0). The latter possibility is supported by the fact that in this work the direct use of $\Delta\phi_Q^F$ has apparently sufficed to specify Q^- as a substrate, while various choices of values for p and c to calculate Q^- led to no simple dependence of the intensity of delayed light on ϕ^F. In any event the variability of the delayed emission yield with Q^- is very small.

A conclusion of little or no energy transfer is in conflict with the earlier work of Joliot and of Delosme, but these authors have now proposed an entirely new model of Photosystem II involving two quenching, primary acceptors which accounts for the kinetic characteristics of the fluorescence rise without requiring significant energy transfer between units[19,23,24]. If extension to other time ranges is permissible the lack of influence of ϕ^F as an emission factor in delayed light emission is of some importance in interpreting many existing publications. For example Mayne[25], Clayton[2], and many others[26–28] have concluded, by using ϕ^F or $\Delta\phi^F$ in Eqns 2 or 4, that J decays with second-order kinetics. Now it may be seen that J varies directly as Z^+, (providing k in Eqns 8–10 does not change) and the two most likely candidates for Z^+, S_3 and S_2, have both been shown to decay with second-order kinetics by independent means[29,30].

The actual value of the emission yield for delayed light may, perhaps, be approximated by the open unit or minimum, "live" prompt fluorescence yield. With the question of "dead" fluorescence still not settled (Clayton's results can just as well be explained by a single dependence on $\Delta\phi^F$ as indicator of Q^-) the value of the minimum prompt fluorescence yield is also uncertain. Surprisingly, wide variations in the absolute value of the delayed emission yield have little effect on calculations of energy barriers from saturation intensities such as done by Zankel[21].

APPENDIX

The following, elegant derivation is due to Dr H. A. Otten, my own being considerably more labored.

A photosynthetic unit, consisting of bulk chlorophyll associated with a single photochemical reaction center or trap, is considered to have three dissipation routes for singlet excitation energy:

(1) transfer to a neighboring unit of randomly determined type (open or closed): probability of transfer *from* an open unit $= s$; probability of transfer *from* a closed unit $= p$;

(2) photochemical utilization (only in an open unit): probability of photochemical utilization $= c$;

(3) radiative and non-radiative loss: total probability of radiative and non-radiative decay $= l_0$ (in open unit) and l_c (in closed unit).

But $p + l_c = 1$ for closed unit and $c + s + l_0 = 1$ for open unit, thus $s = p(1-c)$, $l_c = 1-p$, $l_0 = (1-p)\cdot(1-c)$.

For random excitation of a large number of units, which is the case for excitation by incident light, the total loss yield

$$\psi^F = q\cdot l_0 + (1-q)\cdot l_c + q\cdot s\cdot\psi^F + (1-q)\cdot p\cdot\psi^F$$

where q is the proportion of open units. By substitution for s, l_0 and l_c one obtains:

$$\psi^F = \frac{(1-p)(1-qc)}{1-p(1-qc)}$$

If the rates of radiative and non-radiative decay are constant, which is necessarily assumed to make analysis at all possible, then the fluorescence yield, ϕ^F, is given by:

$$\phi^F = \psi^F\cdot\phi^F_{max}$$

Considering the origin of the individual excitations upon absorption of incident quanta:

$$\psi^F = q\cdot\psi^F_{open} + (1-q)\cdot\psi^F_{closed}$$

where ψ^F_{open} is the total loss yield for excitation initially (before any transfer) in open units and ψ^F_{closed} is for excitation initially in closed units. If delayed light is produced by a back reaction then the excitation must arise in an open unit (*i.e.* the probability factor, q, is unity) and the loss yield applicable is ψ^F_{open}; but

$$\psi^F_{open} = l_0 + s\cdot\psi^F$$

$$(\text{and } \psi^F_{closed} = l_c + p\cdot\psi^F)$$

hence:

$$\psi^F_{open} = \frac{(1-p)(1-c)}{1-p(1-qc)}$$

$\phi^F_{open} = \psi^F_{open}\cdot\phi^F_{max}$ is thus the luminescence emission yield, ϕ^L, used in the Introduction.

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