THE RELATIONSHIP BETWEEN THE YIELD FACTORS FOR PROMPT AND DELAYED FLUORESCENCE

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1. Introduction

Lavorel was the first to emphasise the possible importance of the prompt chlorophyll fluorescence yield on the intensity of delayed fluorescence emission from photosynthetic systems [1]. By analogy with the relation between incident light intensity I and prompt chlorophyll fluorescence intensity F,

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

where Φ_F is the prompt fluorescence yield, Lavorel suggested that the delayed fluorescence intensity L should be described by a similar relationship;

$$L = \Phi_{DF} J \quad (2)$$

where J is the rate of chlorophyll singlet formation and Φ_{DF} is the fluorescence yield of the chlorophyll molecules through which the delayed fluorescence exciton migrates before de-excitation. To understand the relationship between Φ_{DF} and Φ_F has important implications since it would reveal information about the extent of the migration of an exciton away from the trap from which it originated [2–5]. Unfortunately earlier studies designed to investigate the relationship between Φ_{DF} and Φ_F have not been entirely satisfactory since there are a number of factors which can affect J and which need to be controlled (see [4]). A useful expression to describe the rate of chlorophyll singlet excitation which gives rise to delayed fluorescence was formulated by Crofts et al. [6] based on the results of several workers and can be written:

$$J = (Z^+ \text{ chl } Q^-)\, k'\, \nu \exp\left[-(E_{ac} - \Delta p)/kt\right] \quad (3)$$

where $(Z^+ \text{ chl } Q^-)$ is the concentration of the charge transfer complex generated in the reaction centres of photosystem two (PS2), thought to act as the precursor for delayed fluorescence, k' is a constant containing entropy terms, ν is a frequency factor, E_{ac} is the activation energy for the back reaction and Δp is the high energy state expressed as Mitchell's protonmotive force given by

$$\Delta p = \Delta\psi + 2.303 \frac{RT}{F} \Delta\text{pH} \quad (4)$$

where $\Delta\psi$ is the electrical gradient and ΔpH is the pH gradient across the thylakoid membrane and the other symbols have their usual meanings. Combining eq. (2) and eq. (3)

$$L = \Phi_{DF} (Z^+ \text{ chl } Q^-)\, k'\, \nu \exp\left[-(E_{ac} - \Delta p)/kt\right] \quad (5)$$

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea

The object of the work presented in this paper was to compare changes in prompt and 1 ms delayed fluorescence under conditions when the factors controlling J were kept constant. To do this we have taken advantage of the fact that the prompt fluorescence yield can be varied independently of the redox state of PS2 by subjecting isolated, broken chloroplasts to media containing different levels of metal cations. The effect of metal cations on chlorophyll fluorescence has been studied extensively since the phenomenon was first reported by Homann [7] and Murata [8] and seems to involve changes in the electrical field or positive space charge density immediately adjacent to the thylakoid membrane surface [9,10].

2. Materials and methods

Intact chloroplasts were isolated from one-week old pea seedlings as described previously [11]. Prior to experimenting, an appropriate quantity of stock chloroplasts were subjected to osmotic shock with 1.5 ml water in a 1 cm cuvette and immediately made up to 3 ml with double-strength buffer. The chlorophyll concentration was 10 $\mu g.ml^{-1}$ and final suspension medium, except where stated, was 0.33 M sorbitol and 0.01 M *N*-2-hydroxymethylpiperazine–*N'*-2-ethanesulfonic acid (Hepes) brought to pH 7.6 with Tris base. The intensities of 1 ms delayed and prompt fluorescence were measured using a phosphoroscope as described earlier [12]. Actinic illumination was from a tungsten/iodine lamp via a 4 mm Schott BG18 and Balzer Calflex C filter at an intensity of 40 $J.m^{-2}.s^{-1}$ and the emission monitored by two EMI 9558B photomultipliers shielded by a Balzer B40 694 and 2 mm Schott RG695 filter combination for prompt fluorescence and by a Schott RG695 filter for the delayed emission. Chlorophyll concentrations were determined by the method of Arnon [13]. In all experiments, the chloroplasts were treated with 10^{-7} M gramicidin and 6.6×10^{-5} M DCMU.

3. Results

The effect of adding gramicidin and DCMU was to substantially reduce the intensity of 1 ms delayed fluorescence, often by as much as fifty times (see ref. [14]) while prompt fluorescence was maintained at a maximum level as expected for the condition when the photosystem two (PS2) acceptor Q is kept fully reduced by the actinic illumination. Under these conditions when the Mg^{2+} concentration of the suspending medium was raised the yield of the chlorophyll fluorescence increased with $C_{1/2}$ of about 0.4 mM. As shown in the insert of fig.1B, simultaneous measurements of 1 ms delayed fluorescence showed the signal to consist of a fast spike seen during the first few seconds of illumination followed by a settling down to a relatively steady-state level (S level). The properties of the initial spike have not been studied because of the time resolution of the apparatus and we have investigated the effect of cations on the steady-state level (S level) of the delayed emission. As can be seen in fig.1B delayed fluorescence, like prompt

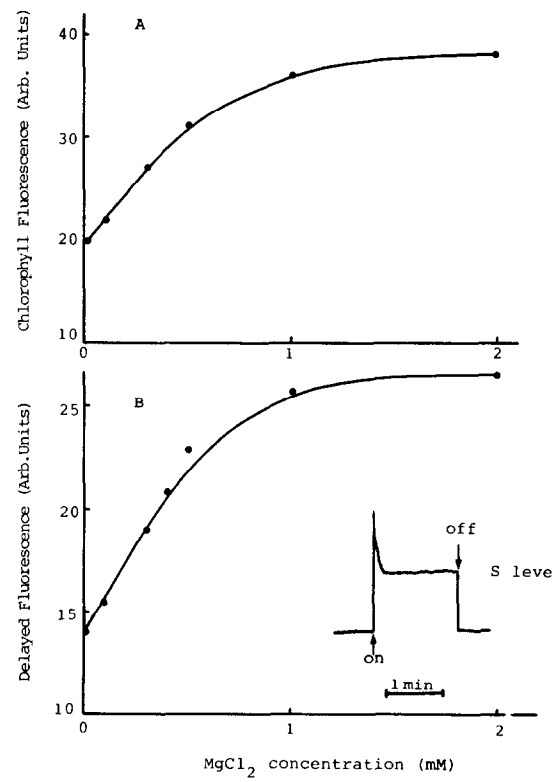

Fig.1. Effect of various levels of $MgCl_2$ on the ability of prompt and 1 ms delayed fluorescence. The insert in B shows a typical 1 ms delayed light signal.

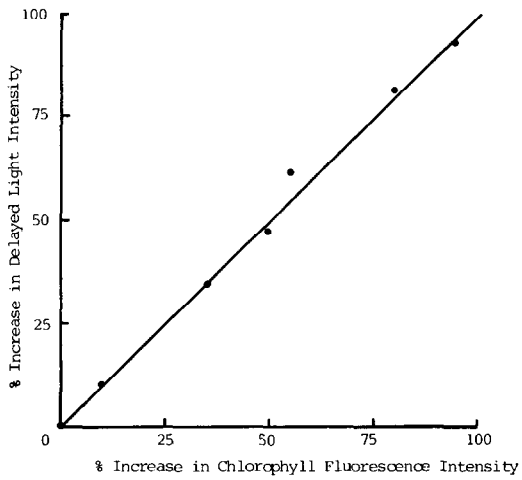

Fig.2. Comparison of the Mg^{2+}-induced change of prompt and delayed fluorescence using the data of fig.1.

fluorescence, increased its yield with increasing concentrations of Mg^{2+}. When the changes in the total prompt fluorescence yield Φ_F (including both the variable Φ_v and the background fluorescence corresponding to dark-adapted, fully-open trap conditions, Φ_o) is used then there is a close relationship between the changes in the two yield factors (see fig.2). The Mg^{2+}-induced yield changes were essentially independent of the anion used and, as shown in table 1, when other alkaline earth cations were used there was again a good correlation between Φ_{DF} and Φ_F. However when Mn^{2+} was used the increase in prompt fluorescence yield was greater than that observed with the delayed emission.

As originally shown by Murata [8,15] the addition

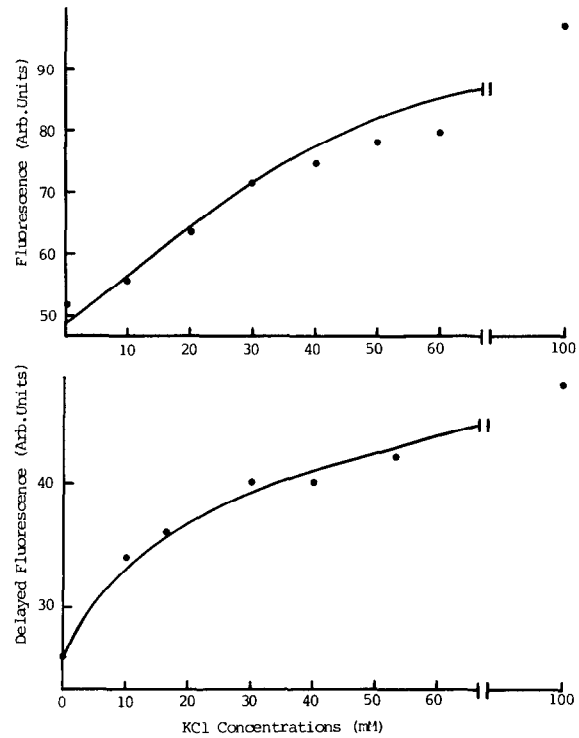

Fig.3. Effect of various levels of KCl on the intensity of prompt and 1 ms delayed fluorescence.

Table 1
Effect of different divalent cations on prompt and ms delayed fluorescence

	Control	Cation added (3 mM)[a]				
		Mg	Ca	Ba	Sr	Mn
Prompt fluorescence (arb. units)	3.5	7.1	6.7	7.0	6.7	6.7
% Change	100	203	191	200	191	194
Delayed fluorescence (arb. units)	4.4	8.5	8.7	9.0	8.9	6.5
% Change	100	193	197	204	206	148

[a] Salts added as chlorides
Results are mean of at least three determinations for each treatment

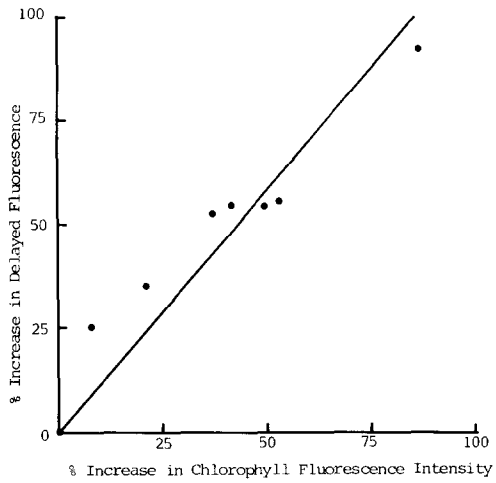

Fig.4. Comparison of the K^+-induced change of prompt and delayed fluorescence using the data of fig.3.

of monovalent cations can also induce changes in the yield of prompt fluorescence from DCMU-poisoned chloroplasts. Again, as fig.3 and fig.4 show, there was a reasonably good correlation between Φ_{DF} and the total fluorescence yield factor Φ_F and again this relationship held for a number of alkali metal cations (see table 2).

Gross and Hess [16] and others [9,17] have shown that if DCMU-treated, broken chloroplasts are suspended in a cation-free medium then the prompt fluorescence is initially high and can be decreased by additions of low levels of monovalent cations (5–10 mM). The prompt fluorescence can be increased back to the original level by further addition of high concentrations of monovalent cations. Such an effect is shown in fig.5A; however the changes observed in the prompt fluorescence yield did not strictly correlate with the changes in yield of ms delayed emission measured under the same conditions (see fig.5B).

4. Discussion

Under a variety of conditions there seems to be a close correlation between Φ_{DF} and Φ_F, where $\Phi_F = \Phi_v + \Phi_o$ (Φ_v is the yield of variable fluorescence and Φ_o is the fluorescence yield corresponding to fully open traps). This finding contrasts with the conclusions of previous workers who have concluded that the delayed fluorescence yield factor Φ_{DF} is independent of the macroscopic prompt fluorescence yield [3] or is dependent only on the variable prompt fluorescence yield [2,5]. This discrepancy can partly be explained because of the problem of maintaining the back reaction J constant. It is this problem which is offered as an explanation for a breakdown in the relationship between Φ_{DF} and Φ_F under some conditions used above. For example, when chloroplasts are suspended in the Gross and Hess medium, which contains no cations, it is known that electron transport can be modified [18]. Changes in J could reflect changes in the concentration of Z^+. Wraight [3] and more recently Malkin [5] have found that L is

Table 2
Effect of different monovalent cations on prompt and ms delayed fluorescence

	Control	Cation added (100 mM)[a]			
		Na	K	Rb	Cs
Prompt fluorescence (arb. units)	4.6	8.3	8.3	8.4	8.3
% Change	100	180	180	183	180
Delayed fluorescence (arb. units)	3.1	5.4	5.5	6.1	6.0
% Change	100	174	177	196	192

[a] Salts added as chlorides
Results are the mean of at least three determinations for each treatment

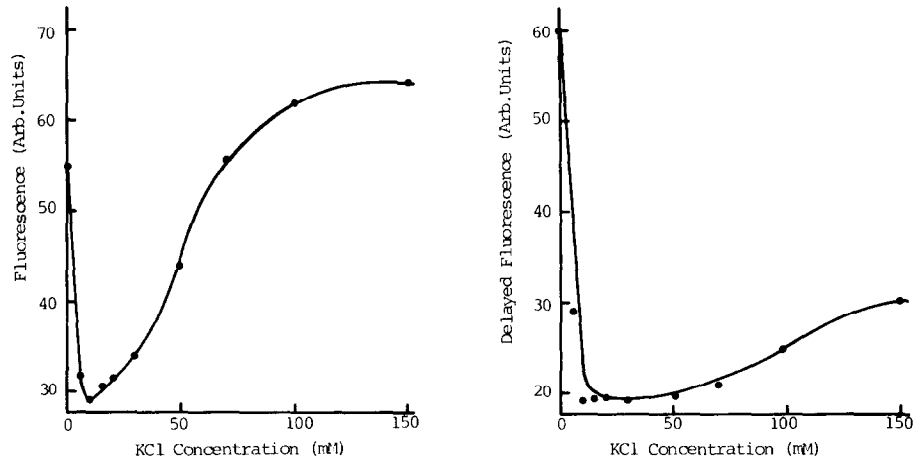

Fig.5. Changes in the intensity of prompt and 1 ms delayed fluorescence on addition of KCl to broken chloroplasts treated with 10^{-7} M gramicidin and 6.6×10^{-5} M DCMU suspended in a cation free medium consisting of 0.1 M sorbitol plus sufficient Tris base to bring the pH to 7.0.

linearly related to (Q^-) and not to $(Q^-)^2$ as predicted by eq. (5). It was from this observation that Wraight concluded that there was no relationship between Φ_{DF} and Φ_F while Malkin argued that (Q^-) was not the precursor for delayed fluorescence but that Φ_{DF} was related to Φ_V.

We concluded that if sufficient care is taken to maintain J constant then an exciton created by a back reaction in a PS2 trap is able to migrate into the light-harvesting chlorophyll antenna and has the same properties as an exciton generated by direct light absorption. Such a conclusion suggests that the open reaction centre generated by the back reaction is either not a perfect trap or that there is sufficient lag before it is able to trap again. Either case would allow the exciton to diffuse into the bulk chlorophylls. Moreover the possibility of rapid exciton migration away from a non-perfect trap would account for the existence of Φ_o and explain why Φ_{DF} is related to the total fluorescence yield Φ_F. Finally it should be noted that the conclusion that Φ_{DF} equals Φ_F has been found under conditions when the majority of PS2 traps are closed.

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References

[1] Lavorel, J. (1968) Biochim. Biophys. Acta 153, 727–730.
[2] Clayton, R. K. (1969) Biophys. J. 9, 60–76.
[3] Wraight, C. A. (1972) Biochim. Biophys. Acta 283, 247–258.
[4] Lavorel, J. (1975) in: Bioenergetics of Photosynthesis. (Govindjee ed) pp. 223–317, Academic Press.
[5] Malkin, S. (1976) in: Proc. VII Int. Congr. Photobiol., Rome.
[6] Crofts, A. R., Wraight, C. A. and Fleischman, D. E. (1971) FEBS Lett. 15, 89–100.
[7] Homann, P. (1969) Plant Physiol. 44, 932–936.
[8] Murata, N., Tashiro, H. and Takamiya, A. (1970) Biochim. Biophys. Acta 197, 250–256.
[9] Barber, J. and Mills, J. (1976) FEBS Lett. 68, 288–292.
[10] Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 74, 174–181.
[11] Telfer, A., Barber, J. and Nicolson, J. (1975) Plant Sci. Lett. 5, 171–176.

[12] Barber, J. (1972) Biochim. Biophys. Acta 275, 105–116.
[13] Arnon, D. I. (1949) Plant Physiol. 24, 1–15.
[14] Hipkins, M. F. and Barber, J. (1974) FEBS Lett. 42, 289–292.
[15] Murata, N. (1971) Biochim. Biophys. Acta 226, 422–432.
[16] Gross, E. L. and Hess, S. C. (1973) Arch. Biochem. Biophys. 159, 832–836.
[17] Vandermeulen, D. L. and Govindjee (1974) Biochim. Biophys. Acta 368, 61–70.
[18] Walz, D., Schuldiner, S. and Avron, M. (1971) Eur. J. Biochem. 22, 439–444.