# DOES THE ACCEPTOR $Q_2$ FULFIL AN INDISPENSABLE FUNCTION IN THE PRIMARY REACTIONS OF PHOTOSYSTEM II?

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Received 17 August 1981

## 1. Introduction

In the presence of the inhibitor DCMU, fluorescence induction in chloroplasts and algae reflects the reduction of Q, the primary acceptor of PSII [1]. It is clear from detailed studies of fluorescence induction that Q shows several kinds of heterogeneity:

(i) Redox titration of the fluorescence yield indicates the existence of two Q components with $E_{m,7} \sim 0$ mV ($Q_H$) and −250 mV ($Q_L$) [2–6];

(ii) Whereas ~70% of the variable fluorescence can be removed by photoreduction with a single saturating flash (the Q reduced is termed $Q_1$) the remainder is removed only after several such flashes (the inefficiently reduced Q is called $Q_2$) [7–9];

(iii) The fluorescence induction curve under continuous light can be resolved into a fast, sigmoidal phase and a slow exponential one, which are considered to be due to closure of $PSII_\alpha$ centres and $PSII_\beta$ centres, respectively [10–12]. $PSII_\alpha$ and $PSII_\beta$ have different antenna size but are reduced with the same quantum efficiency [13,14]. A major problem has been to understand the relationships between these Q 'components' and to explain their function in photosynthesis (see, e.g., [15]).

Both $PSII_\alpha$ and $PSII_\beta$ are associated with the C550 absorbance change and (from measurement at 320 nm) with quinone-type acceptors [16,17]. Therefore, because $Q_2$ is not associated with C550 [8,9], it cannot merely be explained as due to the presence of $PSII_\beta$. Reduction of $Q_1$ is accompanied by reduction of C550 and hence it could be the acceptor of $PSII_\alpha$. Redox titration has revealed that the acceptor of $PSII_\beta$ titrates with an $E_{m,7} \simeq +120$ mV [18,19] and therefore cannot be either $Q_L$ or $Q_H$ which both appear to be associated with $PSII_\alpha$ [20]. Only $Q_H$ is associated with C550 [21]. Therefore, it has been suggested that $Q_H$ may be identical to $Q_1$ and $Q_L$ may be $Q_2$ [15], but no direct experimental evidence exists to substantiate this. To make progress in this area it is necessary to combine potentiometric and kinetic approaches utilizing experimental systems which show different proportions of the various Q components [18,20]. Here, we describe a system from a blue-green alga in which only one quenching species, which titrates as a single redox component, is present. Evidence is provided to support the above notion that $Q_1 = Q_H$ and $Q_2 = Q_L$ and to indicate that photosynthetic electron transport from $H_2O$ through PSII does not require $Q_2$ as an obligatory electron carrier.

*Abbreviations:* PSII, photosystem II; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; $F_o$, minimum fluorescence when Q is oxidised; $F_m$, maximum fluorescence when Q is reduced; $F_i$, initial fluorescence; $F_i = F_o$ when Q is oxidised

## 2. Methods

Photosystem II particles were prepared from *Phormidium laminosum* essentially as in [22].

Intact chloroplasts were prepared from spinach as in [24], and osmotically shocked immediately prior to use.

Redox titrations were performed as in [4,18] using the following mediators: 1,2-naphthoquinone (10 μM), 5-hydroxy-1,4 naphthoquinone (10 μM), duroquinone (10 μM), pyocyanine (10 μM), indigotetrasulphonic acid (20 μM), 2-hydroxy-1,4 naphthoquinone

*Published by Elsevier/North-Holland Biomedical Press*
00145793/81/0000–0000/$02.75 

(15 μM), anthraquinone (15 μM), anthraquinone-2-sulphonate (15 μM) and anthraquinone-2,6-disulphonate (15 μM). Although the bound lauryldimethylamine oxide present in the PSII preparation can be reduced irreversibly to the tertiary amine by dithionite [23] there was no indication that such a process was occurring or interfering with the results. The particles were suspended in the following medium, at pH 7.5; 25% glycerol, 10 mM $MgCl_2$, 10 mM Hepes–NaOH plus 5 mM phosphate buffer. DCMU was added at 10 μM. Spinach chloroplasts were suspended in the following medium, at pH 7.6: 0.33 M sorbitol, 2 mM EDTA, 5 mM $MgCl_2$ and 50 mM Hepes.

Chlorophyll fluorescence was monitored at 695 nm as in [18,20], except that a bifurcated fibre optic was used and induction curves were stored and processed in a Datalab DL401 analyser and associated software. Single saturating flashes were provided by an Applied Photophysics repetitive flash unit, consisting of 2 flash-lamps mounted at either side of the 4-sided cuvette. The flash rose to a peak at 3 μs and had a width of 5.5 μs at 50% maximum intensity. The flash was unfiltered and was tested for saturation using Balzars neutral density filters.

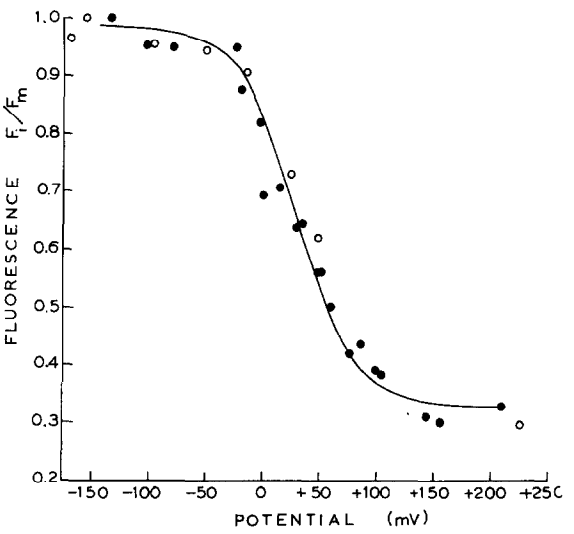


Fig.1. Redox titration of initial fluorescence in *Phormidium* PSII particles: chl *a* was 5.3 μg/ml; (○) oxidative titration; (●) reductive titration. Curve shows Nernst equation ($n$ = 1), $E_m$ + 30 mV. Conditions as in section 2.

## 3. Results and discussion

Previous measurements of chlorophyll *a* fluorescence in blue-green algal cell-free preparations have been hampered by the presence of functionally dissociated phycocyanin which is also highly fluorescent in the same region (685–695 nm) [25,26]. The use of a photosystem II particle from which the major fraction of the phycocyanin pigment has been removed without loss of photochemical activity [22,27] reveals a fluorescence induction curve remarkably similar to that observed in higher plants and green algae in terms of yield per chlorophyll, extent of variable ($F_v$) to fixed ($F_o$) fluorescence, and degree of sigmoidicity (fig.2). (We will be discussing sigmoidicity in a forthcoming article.)

Fig.1 shows a titration of $F_i/F_m$ using *P. laminosum* PSII particles, obtained by measuring fluorescence induction curves at a variety of redox potentials. The titration was fully reversible and comprises a single component with $E_{m,7.5}$ = +30 mV. There is no evidence for the lower potential component ($Q_L$) which titrates with $E_{m,7} \sim -250$ mV ($n$ = 1) in higher plants [2–6,19] or $E_{m,7.5} \sim -100$ mV ($n$ = 1) in photosystem II particles prepared from *Chlamydomonas reinhardtii* [28] (J. M. B., unpublished).

In [7,9], the number of single-turnover saturating flashes given to chloroplasts which are required to attain the maximal yield of fluorescence have been measured in the presence of hydroxylamine and DCMU. One flash produces ~75% $F_{max}$ and the remaining quenching is gradually removed by ~4 subsequent flashes and is attributed to the presence of $Q_2$. The charge stabilisation in centres with $Q_2$ is less efficient than in centres with $Q_1$ as a primary acceptor. We have repeated this experiment using chloroplasts (as a control) in which both pairs $Q_H$ and $Q_L$ and $Q_1$ and $Q_2$ are known to be present, and *Phormidium* particles in which we have shown that $Q_L$ is absent (fig.1). The results are shown in fig.2.

The sample was dark adapted for 10 min then 10 mM hydroxylamine and 10 μM DCMU added. A single saturating flash was given and a fluorescence induction curve measured 10 ms later.

According to [7], in the presence of hydroxylamine, predominantly single hits occur during the flash, the small proportion of double hits being dependent on the concentration of hydroxylamine (which re-reduces the secondary donor D) and on the flash length. Under the conditions of our experiments,

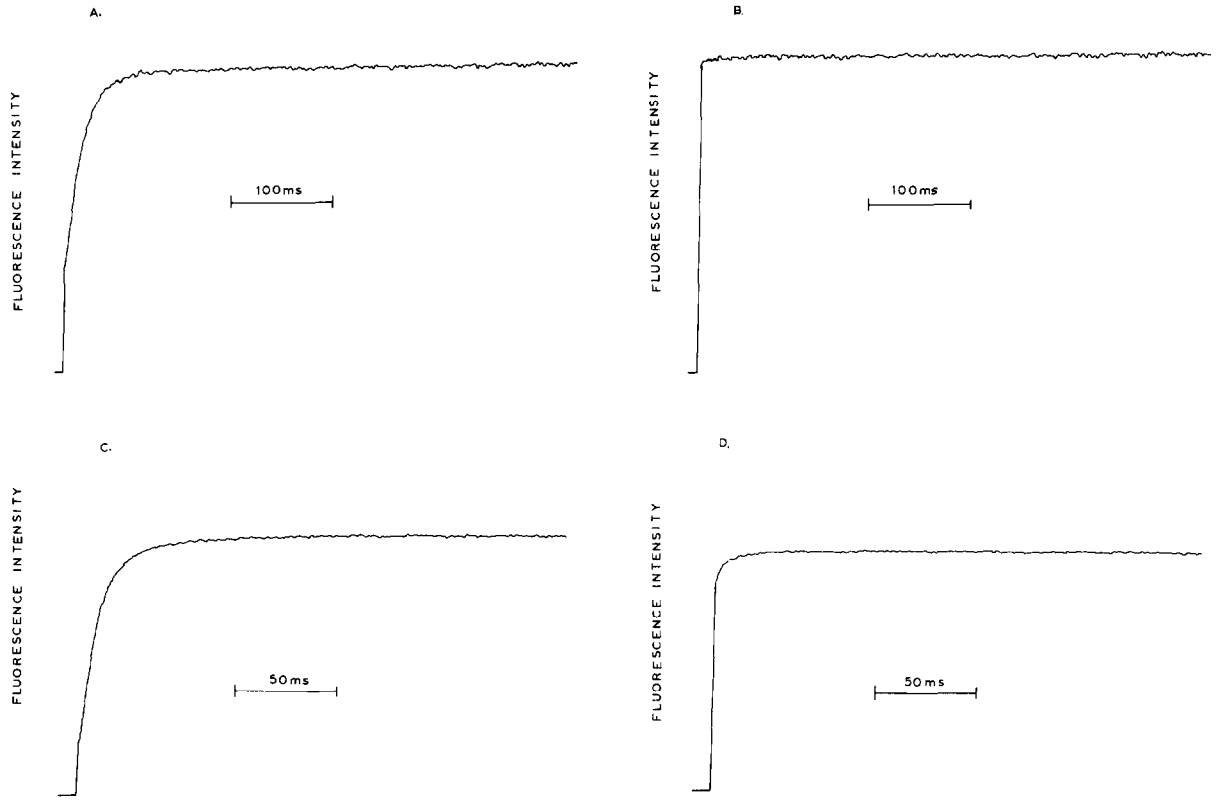


Fig.2. Fluorescence induction in *Phormidium* PSII particles and spinach chloroplasts. Particles or chloroplasts were dark adapted for 10 min and 10 mM hydroxylamine followed by 10 μM DCMU added. Induction curves were then recorded either with or without a saturating flash given 10 ms earlier: (A) particles (control); (B) particles after a saturating flash; (C) chloroplasts (control); (D) chloroplasts after a saturating flash. Chl was 5.3 μg/ml in (A,B) and 52 μg/ml in (C,D), chosen to allow clear resolution of $E_i$ with the same measuring light intensity.

we expect a slightly larger fraction of double hits to occur due to:

(i) The concentration of hydroxylamine used [8,9];

(ii) The flash length.

Fig.2 shows that 3–5% of the variable fluorescence remains quenched in the *Phormidium* particles following a single flash, compared with 20% in spinach chloroplasts. The remaining quenching observed in the particles may be due to either a back reaction during the 10 ms between the flash and fluorescence detection or perhaps to a residual fraction of $Q_L$, but in either case it is clear that a significantly greater fraction of the fluorescence quenching has been removed by a single flash in *Phormidium* than in spinach chloroplasts.

In conclusion, the observations that the initial fluorescence titrates as a single component, and that a single flash is required to reach $F_m$ together suggest that $Q_H$ is the same component as $Q_1$. As a corollary, we also suggest that $Q_2 = Q_L$. A low potential for $Q_2$ is implicit in the suggestion [9] that $Q_2$ can reduce cytochrome *b*-563 which has $E_m = -110$ mV [29].

Finally, these experiments show that electron transport through PSII can occur normally with a relatively simple acceptor system. This suggests that some of the complications observed in chloroplasts are associated with structural or organisational complexities absent in a blue-green alga. This suggestion is consistent with the absence of $Q_L$ in agranal chloroplasts developed under intermittent light [30] and a decrease in proportion of $Q_L$ after depletion of $Mg^{2+}$ from chloroplasts [20].

## Acknowledgements

This research was supported by grants from the UK Science Research Council to P. H. and D. S. B.

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