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Minireview

Period four oscillations in chlorophyll *a* fluorescence

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Abstract

The discovery of period four oscillations of the fluorescence yield under flashing light demonstrated that not only the redox state of the Photosystem II (PS II) electron acceptor Q_A, but also the oxygen evolving cycle (described by the S states) modulates the fluorescence yield of chlorophyll (Chl). The positive charges accumulated on the donor side of PS II act on the fluorescence yield (measured in the Q_A^- state during a strong flash) through the concentration of the quencher P_{680}^+, the oxidized form of PS II reaction center Chl *a*. However, the period four oscillations of the fluorescence yield detected 1 s after a strong flash (in the $P_{680}Q_A$ state) have not yet been fully explained.

Abbreviations: PS II – Photosystem II; P_{680} – reaction center Chl *a* of PS II; S states – redox states of the oxygen evolving complex of PS II; Q_A – the first quinone electron acceptor of PS II

Introduction

The 1963 experiment of L.N.M. Duysens and H.E. Sweers

A short time after the discovery of two photosynthetic systems acting in series (see J. Myers, and J.M. Anderson, this issue), Duysens and Sweers (1963) attempted to explain the induction kinetics of fluorescence by the interaction of the two photosystems. They showed that alternating actinic light 2 (exciting mainly Photosystem II) and 1 (exciting mainly Photosystem I) rapidly increased and decreased the fluorescence yield detected by a weak modulated beam. To explain this finding, they assumed that a redox intermediate in the electron transfer chain between the two photosystems quenched the fluorescence in the oxidized state, but not in the reduced state. This intermediate, closely associated with the reaction center of PS II, was called Q [see also Duysens (1989) for a historical account].

Following this seminal work of Duysens and Sweers, the fluorescence yield of chlorophyll *in vivo* was considered to depend essentially on the redox state of the PS II electron acceptor Q (later named Q_A) (see Figure 1 for a photograph of Duysens.) However, it was not long before this attractive simplicity encountered some notable exceptions.

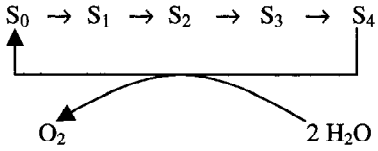
The 1969 experiment of P. Joliot and coworkers, and the 1970 interpretation of B. Kok and coworkers

In 1969, Pierre Joliot and his coworkers measured the oxygen evolution from dark-adapted green algae or isolated chloroplasts upon illumination by a sequence of short saturating flashes (2 μs duration, 0.3 s dark interval). They observed that the amount of oxygen produced by the 1st, 2nd, ... *n*th flash displayed remarkable arithmetic properties: it oscillated with a periodicity of four flashes. Bessel Kok et al. (1970) interpreted this result by a linear four-step mechanism

Figure 1. Louis N. M. Duysens.

Figure 2. Pierre Joliot. Photograph by Govindjee.

of charge cooperation in the oxygen-evolving system:



In the dark, the S_0 and S_1 states are stable, while S_2 and S_3 deactivate towards S_1 in the minute time range. [For historical accounts, see Joliot (1993) and George Cheniae (1993); see Figure 2 for a photograph of one of the authors (P. Joliot); a photograph of Bessel Kok is in the perspective of J. Myers, this issue.]

Chlorophyll fluorescence yield in the Q_A^- state

A short time later, René Delosme (1971a, b) observed that the fluorescence emitted during the 1st, 2nd, ... *n*th saturating flash of a sequence (dark interval: 1 s) also oscillated with a periodicity of four flashes (maxima for the flash numbers 1, 5, 9, etc., and minima for 3, 7, 11, etc.) and correlated with the sum $[S_2]+[S_3]$. Figure 3 shows a photograph of one of us (René Delosme) and Figure 4 shows the data. Since in the considered time window, the PS II electron acceptor was entirely in the reduced form Q_A^-, this experiment proved that the amount of fluorescence emitted during a saturating flash depended not only on the redox state of Q_A, but also on a 'second quenching process' related to the oxidation states of the PS II electron donor (S states).

Interpretation

On the basis of fluorescence kinetics at 77 K, S. Okayama and Warren Butler (1972) suggested that the primary electron donor of PS II in the oxidized state (P_{680}^+), as well as the electron acceptor Q_A, could quench the fluorescence. The quenching by P_{680}^+ could also explain (Butler 1972) the 25 ns fluorescence rise (much slower than the reduction of Q_A), which, according to David Mauzerall (1972), followed a single saturating flash of 10 ns. The 25 ns rise then reflected the reduction of P_{680}^+ in the dark. [See also A. Sonneveld et al. (1979) and the historical viewpoint of Govindjee (1995).]

Duysens et al. (1975) were the first to precisely state that P_{680}^+ was the 'second quencher' revealed by the fluorescence oscillations in the Q_A^- state. E. Schlodder et al. (1985) confirmed that the amount of P_{680}^+ present in the microsecond range oscillates with a periodicity of 4 flashes and follows the sum $[S_2]+[S_3]$. F. Rappaport et al. (1994) attributed these changes to a decrease of the equilibrium constant for $P_{680}^+Y_z \longleftrightarrow P_{680}Y_Z^+$ induced by the $S_1 \rightarrow S_2$ transition. According to these authors, the equilib-

Figure 3. René Delosme.

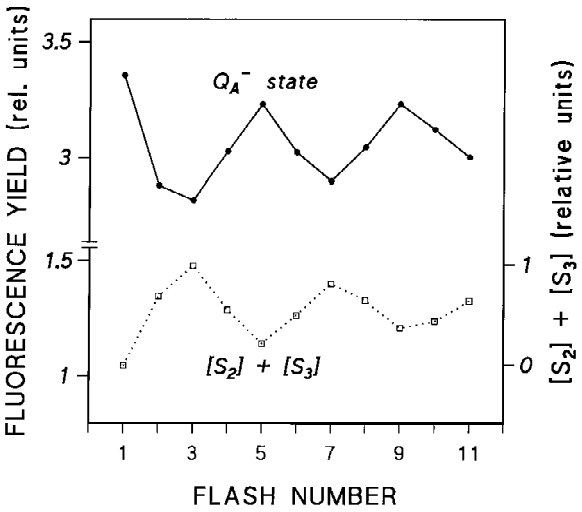


Figure 4. Correlation between the chlorophyll *a* fluorescence yield under strong flashing light and the concentration $[S_2]+[S_3]$ in spinach chloroplasts. The fluorescence emitted during the *n*th strong flash (in the Q_A^- state) correlates with the sum $[S_2]+[S_3]$ just before the *n*th flash, calculated from oxygen measurements of Bouges (1971). Dark time between the flashes: 1 s. From Delosme (1971b).

rium constant is modulated by the net electrostatic charge of the manganese cluster, which depends on the movements of electrons and protons.

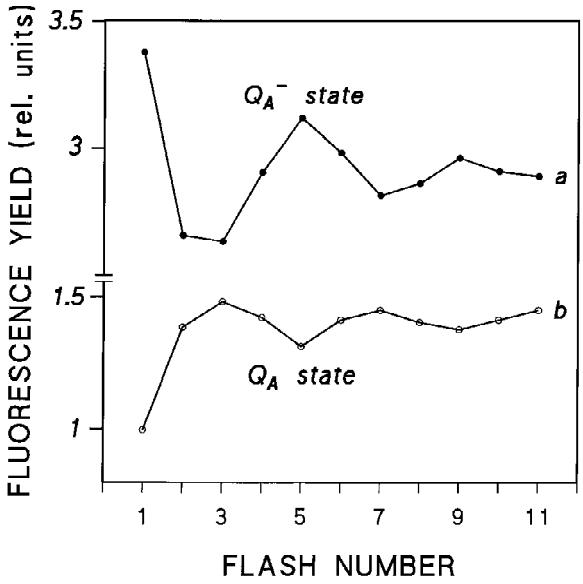


Figure 5. (a) Fluorescence yield measured during the *n*th strong flash (in the Q_A^- state) in *Chlorella pyrenoidosa*. (b) Fluorescence yield measured during a weak detecting flash 10 ms before the *n*th strong flash (Q_A state). Dark time between the strong flashes: 1 s. From Delosme (1971b).

Chlorophyll fluorescence yield in the Q_A state

The fluorescence emission was also measured during a very weak detecting flash, 10 ms before the *n*th saturating flash, after complete reoxidation of Q_A^- (Figure 5). As previously observed under similar conditions by Joliot and his coworkers (Joliot et al. 1971; Joliot and Joliot 1971), oscillations with periodicity of 4 occurred (minimum just before the actinic flashes 1, 5, 9...; maximum just before the actinic flashes 3, 7, 11...) (Figure 5), and the fluorescence yield correlated again with the sum $[S_2]+[S_3]$.

Interpretation

The oscillatory pattern in the Q_A state has not yet been fully explained. The quencher $P_{680}^+$is of little help here, because it has not been detected in significant amounts in the time range of seconds following a saturating flash. Alternatively, the presence of a long-lived positive charge on the donor side of PS II could increase the Chl *a* fluorescence yield when the system is in the S_2 and S_3 states. Consistent with this, Bruce Diner and Joliot (1976) found that the presence of a delocalized transmembrane electrical field blocks some PS II reaction centers in a nonquenching state. Alternative quenching mechanisms (including

the paramagnetism of the S_0 state) have been also discussed by Shinkarev et al. (1997).

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