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S-state dependence of the miss probability in photosystem II

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Introduction

The oxygen production of dark-adapted photosystem II (PS II) upon illumination by a series of single-turnover flashes shows a damped period four oscillation with flash number (Joliot and Kok, 1975). The oscillation implies that PS II cycles through 4 different states (S-states) during the flash series. Its damping is attributed to misses reflecting a non-zero probability that a reaction center fails to contribute to the oxygen evolution process in spite of a saturating excitation. Misses can have various origins, all of which are likely to depend on the state of PS II and hence on flash number in the series. When only a single S-state or transition between two S-states is measured, such as in the flash yield of oxygen production, only the average miss probability during the S-state cycle can be determined (Lavorel, 1976; Thibault, 1978). In general, however, the S-state dependence of misses affects the quantitative interpretation of all phenomena modulated by the period four oscillation and may provide information on the kinetics and thermodynamics of PS II functioning.

The chlorophyll fluorescence yield of PS II provides a convenient and very sensitive tool to monitor electron transfer reactions in PS II (van Gorkom, 1986). In chloroplasts from higher plants, photochemical quenching by open PS II reaction centers decreases the fluorescence yield approximately 5-fold. When the primary quinone acceptor Q_A is reduced, photochemical quenching no longer occurs. The fluorescence yield does not increase, however, unless also the primary donor P_{680} is reduced, because P_{680}^+ is an equally effective fluorescence quencher. Consequently, misses caused by the presence of Q_A^- should be reflected in an enhanced fluorescence yield before the flash, and misses caused by charge recombination before P_{680}^+ is reduced by the secondary electron donor Y_Z should lead to a smaller fluorescence yield increase after the flash. If additional misses are required to explain the damping and period of the oscillation, they may be attributed to later losses by reverse, cyclic, or side reactions that do not contribute to S-state advancement.

Materials and methods

Measurements were performed on thylakoids isolated from laboratory-grown spinach leaves and stored until use in small portions at 77 K in a buffer containing: 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$ and 25 mM Tricine (pH 7.8). Shortly before measurement the thylakoids were diluted from a concentrated suspension to a chlorophyll *a+b* concentration of 15 $\mu g/ml$ in a buffer similar to the storage buffer but containing MES/NaOH (pH 6.5) instead of Tricine.

Fluorescence was excited by 470 nm light-emitting diodes modulated at 2 MHz and measured at 686 nm by a gated photomultiplier and lock-in amplifier as described (de Wijn and van Gorkom, 2001). Saturating 10 ns, 532 nm flashes were obtained from a Nd:YAG laser. The instrument response time was set to 2 μs for the fast measurements and 10 μs for

the slow measurements. For each measured flash series a fresh, dark-adapted sample was taken. Flash series were averaged 3-4 times. All measurements were performed at 20 °C. Least squares fitting of the result of model calculations to the data was performed using the routine e04jaf of the NAG toolbox for Matlab.

Results

Figure 1 shows the flash-induced fluorescence yield changes that were induced in a thylakoid suspension at pH 6.5 by a train of saturating flashes fired at 1 Hz. The measurements were performed on a time scale of 150 μ s (left panel) and 1.6 ms (right panel). After a flash the fluorescence yield rises to 3.5 - 4 times F_0 (the fluorescence yield in the dark-adapted state), much less than the approximately 5 x F_0 observed when all centers are closed (e.g. by a few flashes in the presence of DCMU and TPB, not shown). The flash-induced rise takes place largely within the time-resolution of the measurement, but continues in the first few tens of microseconds after the flash. After that the fluorescence yield decreases in a highly polyphasic manner to values near 1.2 x F_0 just before the next flash, one second later. Both the maximum and the minimum fluorescence yield depend on flash number and oscillate with a periodicity of four.

The period four oscillation of the flash-induced fluorescence yield increase has been known for 30 years (Delosme, 1971; Zankel, 1973). Duysens et al. (1975) already discussed it in terms of slow P_{680}^+ reduction and consequently enhanced loss of the charge separated state by recombination. We recently investigated charge recombination during the slow phases of P_{680}^+ reduction (de Wijn et al., 2001). A considerable fraction of P_{680}^+ reduction takes place with a 20-30 μ s time constant and was found to compete with a 100 - 200 μ s charge recombination. In addition a substantial amount of recombination was found on a flash fired in the S_3 state, attributed to presence of $Y_Z^{ox}S_2$ in equilibrium with Y_ZS_3 . This would largely prevent the reduction of P_{680}^+ by Y_Z , because the reduction of Y_Z^{ox} by S_2 is expected to be

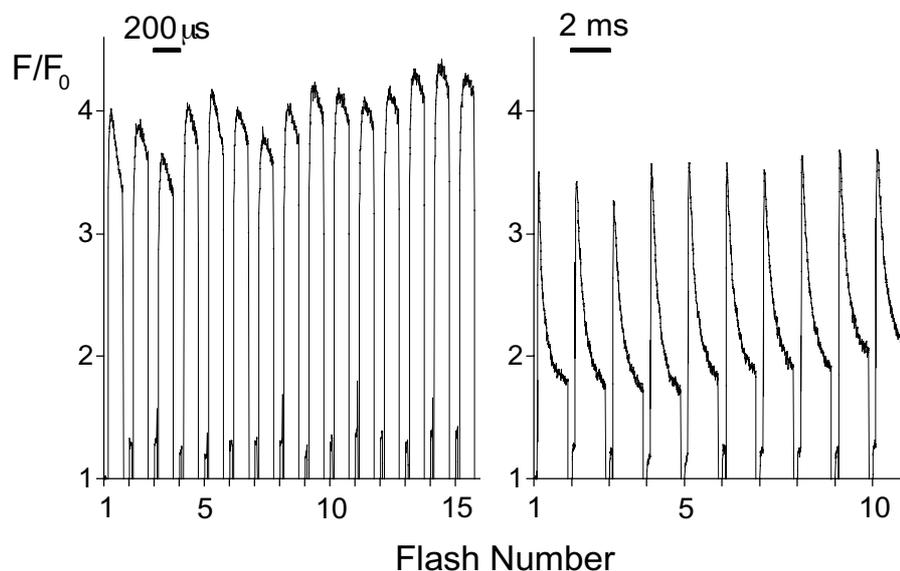


Fig.1. Flash induced fluorescence changes induced by a series of saturating flashes fired at 1 Hz in a thylakoid suspension: Left panel: 150 μ s measurement time, right panel: 1.6 ms measurement time.

slower than $P_{680}^+Q_A^-$ recombination. Both slow phases in P_{680}^+ reduction are expected to cause a fluorescence yield increase that overlaps in time with the fluorescence yield decrease

due to re-oxidation of Q_A^- . To estimate the extent of fluorescence quenching by PS II centers where $P_{680}^+Q_A^-$ recombination occurs, an extrapolation of the later fluorescence yield decay back to the time of the flash is required.

For this purpose a simultaneous fit to both the short and long time scale data in Figure 1 was carried out, using an exponential rise phase, two exponential decay phases and an offset. The kinetics are dependent on flash number (de Wijn and van Gorkom, 2001). In the absence of independent information on rates and amplitudes a unique decomposition of the kinetics is not possible, due to cross-correlation between the parameters. However, the extrapolated value at $t=0$, F_{ex} , is equal to the sum of the amplitudes of the decay phases and the offset and is rather well determined. Assuming that all rise phases in the fluorescence have been adequately accounted for, the value of F_{ex} obtained in this way is a measure of the fraction of centers where the charge separation is eventually stabilized by P_{680}^+ reduction.

Figure 2 shows F_{ex}/F_0 as a function of flash number. Its period four oscillation with characteristic minima after the 3rd and 7th flash indicates that the amount of $P_{680}^+Q_A^-$ recombination depends on S-state and is indeed larger on the S_3 to S_0 transition than on the other transitions. Charge recombination in centers that participate in S-state cycling implies a miss that will contribute to the damping of the oscillation. The oscillation of F_{ex} thus provides a measure both of the average miss probability and of the distribution of miss probabilities over the S-states.

The relation between fluorescence yield and fraction of centers in the non-quenching state depends on three parameters: F_M , F_0' , and p (Paillotin, 1976). F_M is the fluorescence yield observed when all centers are closed. An initial value of $5 \times F_0$ was assumed on the basis of measurements in the presence of DCMU and TPB. The gradual increase of the maximum fluorescence yield during the flash series in Fig.1 is attributed to reduction of the plastoquinone pool, which is a non-photochemical fluorescence quencher in its oxidized state. To account for this increase, the value of F_M was allowed to increase after each flash by a certain fraction that was included as a fit parameter. F_0' is the fluorescence yield observed when all centers are open, which is S-state dependent and generally somewhat higher than F_0 , the fluorescence yield in the dark-adapted state. The actual value of F_0' applicable just after a

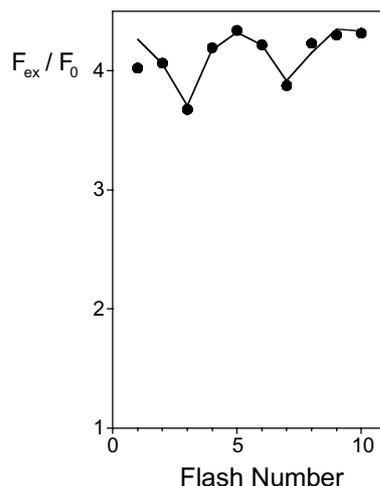


Fig.2. F_{ex}/F_0 as a function of flash number. F_{ex} is obtained by extrapolating the decay of the flash-induced fluorescence changes in Figure 1 back to $t = 0$. The line shows the fit described in the text.

flash is uncertain. We used the fitted amplitude of the offset in the decay kinetics of the fluorescence yield. The connectivity parameter p determines the degree of non-linearity between fluorescence yield and quenching centers: $p=0$ means a linear relation, implying separate PS II units, and $p=1$ means a normal hyperbolic Stern-Volmer relation, implying unrestricted energy transfer between PS II antennae. We used a value of 0.7.

To fit the F_{ex} data in Fig.2 the fractional S-state population was calculated for each flash, starting from an assumed initial distribution of 20% S_0 , 80% S_1 , and four independent fit parameters (m_0 to m_3 in Table 1) for the miss probabilities in the four S-states. The total amount of misses on each flash of the series was translated to fluorescence quenching according to Paillotin (1976) and the result was fitted to the F_{ex} data. The difference between F_M and F_{ex} was too large to be entirely due to recombination in active PS II centers: the implied miss probability would be much larger than the damping and period of the oscillation can explain. A fraction of the centers was therefore assumed to be inactive and to remain in a quenching state throughout the flash series. Since the behavior of inactive centers is often seen to differ on the first flash compared to the later flashes, the point taken after the first flash was not included in the fit.

Table 1.

Miss probability in % obtained by fitting the flash number dependency of F_{ex} (Fig.2).

| | m_0 | m_1 | m_2 | m_3 | m_{av} |
|---------------------|-------|-------|-------|-------|----------|
| $m_Q = 0$ $p = 0.7$ | 0.0 | 5.8 | 8.7 | 18.0 | 8.1 |
| $m_Q = 5$ $p = 0.7$ | 0.0 | 4.3 | 8.7 | 17.0 | 10.0 |
| $m_Q = 5$ $p = 1$ | 0.0 | 3.6 | 4.6 | 10.9 | 7.3 |

m_Q and the $m_{0..3}$ are defined in the text. m_{av} is the resulting average miss probability per flash. Fits were performed for $m_Q = 0$ and $m_Q = 5\%$ respectively and values of the connectivity parameter of 0.7 and 1. For the fits with $p = 0.7$, F_0' was set to 1.4, 1.6, 1.4 and 1.3 for charge separation from the S_0 , S_1 , S_2 and S_3 state, respectively. For the fit with $p = 1$, F_0' was set to 1.2 for all S-states. The fraction of S_0 present after dark adaptation was set to 20 %, the fraction of inactive centers was a fit parameter and amounted to approximately 15 % for all three fits. F_{max} was set to 5 initially and allowed to increase a certain fraction with each flash. This fraction was found to be 0.6 % / flash for all three fits.

A reasonable fit of the data could be obtained in this way (line in Fig.2). Table 1 (with $m_Q = 0$) lists the best-fitting values of the miss probabilities due to charge recombination. However, also misses that do not lead to a lower F_{ex} were expected: centers may be closed at the time of the flash due to the presence of Q_A^- in equilibrium with Q_B^- . A miss probability m_Q was included in the S-states thought to be mainly associated with Q_B^- : S_0 and S_2 . It was taken into account for the S-state progression, but not for the F_{ex} calculation. The best fitting value for m_Q was 0, but the quality of fit was not significantly less when it was fixed to 5%. The m_i resulting in this way are also listed in Table 1. The precise distribution of misses over the S-states also depended on the choices made for F_{max} , F_0' and p . The values in Table 1 should be considered a typical example of the distribution of miss probabilities that can be obtained in this way rather than as an unique solution. Features that are consistently found are the low to absent miss in S_0 , intermediate values (5-10 %) in S_1 and S_2 , and much higher in S_3 . The average value of the miss probability with $m_Q = 0$ was 8.2 %, in agreement with values reported previously. An average value of 10 % results from the fits with $m_Q = 5\%$, which seems a bit too high. It appears that even with a maximum amount of inactive centers (i.e. m_0

= 0) the miss probability implied by the F_{ex} oscillation is rather high compared to previously reported values. The minimum value possible within the present model, for a maximum connectivity ($p=1$) and a minimum $F_0'(1.2)$ was still 7.3 %, with nearly two thirds of the misses caused by $P_{680}^+Q_A^-$ recombination (Table 1).

Discussion

The period four oscillation with flash number of the maximum fluorescence yield reached by a saturating flash indicates that the amount of PS II in which the charge separation is eventually stabilized by reduction of P_{680}^+ varies with S-state. By extrapolation of the subsequent fluorescence yield decay back to $t=0$ and conversion of this value, F_{ex} , to the fraction of centers involved a direct measure of the yield of stabilization in PS II is obtained. This yield is lower than the average miss probability in the oscillation would predict, and in fact barely sufficient to account for the amplitude of the oscillation, even assuming maximum connectivity ($p=1$).

Consequently, the additional difference between F_M and F_{ex} must be attributed to non-oscillating centers and any errors in F_M mainly affect the amount of those centers. Reasonable errors in F_0' do not affect the conclusions; if its actual value shortly after the flash were much higher, even larger misses would be calculated. F_{ex} might be underestimated if the reduction of P_{680}^+ by Y_Z contains a significant component with a lifetime equal to or longer than the fast phase of Q_A^- oxidation. However, we consistently find shorter times for $P_{680}^+Q_A^-$ recombination and a substantial reduction by Y_Z would make the P_{680}^+ lifetime even shorter (de Wijn et al., 2001; de Wijn and van Gorkom, 2001).

The S-state dependence of the miss probability reported here does not exactly match the S-state dependence of the amount of P_{680}^+ present in the microsecond time domain after a flash (de Wijn et al., 2001), which predicted a larger miss probability on S_0 than on S_1 . The rise in F_{max} due to plastoquinone pool reduction was not included in that study and uncertainty about the initial S-state distribution might also play a role.

We conclude that the kinetics of chlorophyll fluorescence yield transients induced by a flash series can be used to estimate the relative amplitudes of the miss probability on each flash. When the plastoquinone pool is oxidized, a major part of the misses must be caused by failure of Y_Z to reduce P_{680}^+ before the charge separation is lost by recombination. The probability of this failure is found to increase with the oxidation state of the oxygen evolving complex.

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