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The involvement of spillover changes in State 1-State 2 transitions in intact leaves at low light intensities

Holger Dau and Ulf-Peter Hansen

Institut für Angewandte Physik, Universität Kiel, Kiel (F.R.G.)

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State 2-to-State 1 transitions in intact leaves adapted to blue light of different intensity were initiated by addition of far-red light. It was found that the f_m-level of chlorophyll fluorescence increases stronger than the f_0-level does. The ratio of the relative changes is about 2 at 1 $W \cdot m^{-2}$ blue light and approaches 1.2 at 10 $W \cdot m^{-2}$. These results indicate the involvement of spillover changes at low light intensities.

If additional far-red light (light 1, $\lambda > 700$ nm) is applied to plants adapted to blue light (light 2, $\lambda < 700$ nm, plant in State 2) oxygen yield increases for about 20 min until a new steady state (State 1) is reached. Since these state transitions have been reported first in 1969 [1] there have been discussions whether the transitions are changes of the so-called 'spillover', i.e., excitation transfer from Photosystem II units to Photosystem I units, or changes of the absorption cross-sections. In vitro (isolated chloroplasts) both mechanisms occur [2,3].

One possible approach for studying this question in vivo is the analysis of the enhancement of the original (f_0) and the maximum (f_m) level of the yield of chlorophyll fluorescence upon addition of light 1. The f_0- and the f_m-levels are the hypothetical levels of chlorophyll fluorescence if the primary electron acceptor is fully oxidised or reduced, respectively. Changes of the absorption cross-section are supposed to influence the f_m- and the f_0-levels of chlorophyll fluorescence to the same extent [2,4,5]. Thus, the f_m/f_0 ratio should remain constant in the case of changes of the absorption cross-section, whereas spillover changes are assumed to change mainly the f_m-level. Recent studies [5,6] on intact leaves suggest that in vivo only changes of the absorption cross-section occur. However, in this article, it is shown that the f_m/f_0-ratio may change upon addition of light 1, depending on light-2 intensity.

Spinach was grown from seeds ('Matador', Nr. 560, Florina, Hamburg) at 10 h light per day of 20 $W \cdot m^{-2}$ intensity at 20 °C, and harvested 6 weeks after sowing. The measurements were performed on dissected spinach leaves with the stem in a water-filled vessel. Light was guided to the leaves via a light guide with four arms connected to the following light sources (feed-back controlled halogen bulbs): (1) measuring light (Schott DAL-filter, 472 nm) chopped with 2 kHz, integrated light intensity of 0.3 $W \cdot m^{-2}$ at surface of the leaf; (2) blue-green (Schott BG 38) actinic light (light 2), intensity as indicated in the text; (3) light 1 (Schott DAL-filter, 720 nm) of intensity of 3 $W \cdot m^{-2}$; (4) the blue-green (Schott BG 38) saturating light pulses (1.2 s duration, intensity of 700 $W \cdot m^{-2}$).

Correspondence: U.-P. Hansen, Institut für Angewandte Physik, Neue Universität, Universität Kiel, Haus N61A, Leibnizstraße 11, D-2300 Kiel, F.R.G.

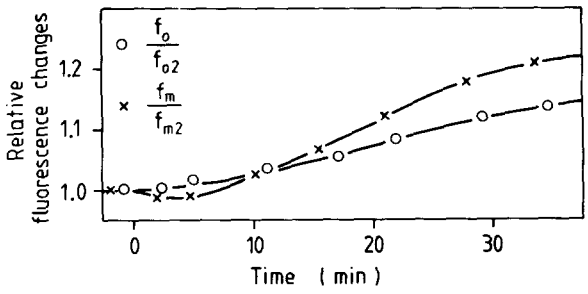

Fig. 1. Time-course of the increase of f_m and f_0 (normalized to the values in State 2) during a State 2-State 1 transition. The leaf has been adapted to a light 2 intensity of 3 $W \cdot m^{-2}$. At $t = 0$ an additional far-red light is applied (3 $W \cdot m^{-2}$).

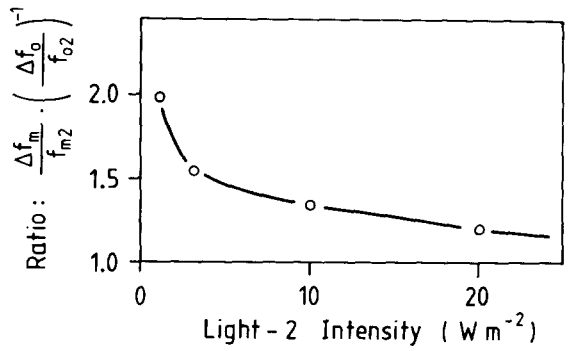

Fig. 2. Intensity dependence of the ratio of the relative changes of f_m and $f_0 = ((f_{m1} - f_{m2})/f_{m2})/((f_{01} - f_{02})/f_{02})$. The measurements are performed on one spinach leaf adapted to the light-2 intensity for 50 min before each measurement.

A fifth arm of the light guide led the fluorescence light to a photodiode detector (filter Schott RG 9). The photodiode detector was connected to a 6th order high-pass filter for proper elimination of non-modulated components. The output signal of the filter amplifier was fed into a lock-in amplifier (Brookdeal, Ortholoc, 9502, integration time constant of 10 ms) referenced by the 2-kHz signal from the chopper. The fluorescence yield as given by the lock-in output signal was recorded on a fast chart recorder.

After adaptation of the leaf to light 2 for half an hour, the State-2 level of f_m (f_{m2}) was determined by application of a saturating pulse [7]. 3 min later the f_0-level (f_{02}) was determined by switching off the blue actinic light and switching on light 1 for 4 s [8]. Our protocol of initiating state transitions followed that of previous workers [1,5,6]: a permanent light 1 in addition to the blue actinic light was applied until a new steady state (State 1, f_{m1}, f_{01}) was reached (10–60 min). The light intensity of 3 $W \cdot m^{-2}$ far-red light is sufficient for a complete transition from State 2 to State 1, in line with findings of other workers [6]. During such a state transition, the f_m- and f_0-levels were determined several times by application of a saturating pulse or switching off the blue actinic light for 4 s, respectively.

Fig. 1 displays the time courses of f_m and f_0 during a State 2-State 1 transition. f_m and f_0 increase with the slow time constant being typical for State 2-State 1 transitions [1,9]. However, the relative increase of f_m is significantly greater than the relative increase of f_0.

In Fig. 2 the dependence of the ratio of the relative f_m changes to the relative f_0-changes on the intensity of the blue light (measured at the steady-state 40–50 min after the change to far-red actinic light, Fig. 1) is shown as measured on one leaf of spinach. A continuous decrease is observed.

TABLE I

f_m AND f_0 CHANGES AT 1 $W \cdot m^{-2}$ AND 10 $W \cdot m^{-2}$ LIGHT 2 INTENSITY

The measurements have been performed on five spinach leaves as described in the text. The standard deviation is given in parenthesis. f_m is the chlorophyll-fluorescence yield obtained from fully reduced primary acceptor, f_0 is that of the fully oxidized primary acceptor. The indices 1 and 2 indicate State 1 and State 2 conditions. The column 'q_E-test' contains the ratio $(f_{m1}/f_{m2})/(f_{m1*}/f_{m2*})$ which is obtained from f_m-measurements with (*) and without preceding dark period, in order to let a possible q_E-quench relax.

	f_{m2}/f_{02}	f_{m1}/f_{01}	f_{m1}/f_{m2}	q_E-test	f_{01}/f_{02}	$\frac{(f_{m1}-f_{m2})/f_{m2}}{(f_{01}-f_{02})/f_{02}}$
1 $W \cdot m^{-2}$ light 2	3.91 (0.23)	4.29 (0.34)	1.20 (0.05)	1.005 (0.005)	1.10 (0.03)	2.14 (0.38)
10 $W \cdot m^{-2}$ light 2	3.40 (0.40)	3.54 (0.35)	1.10 (0.05)	1.004 (0.004)	1.08 (0.03)	1.21 (0.08)

More detailed information about the behavior of the f_m- and f_0-levels of State 2 (f_{m2} and f_{02}) and of State 1 (f_{m1} and f_{01}) measured on five leaves of spinach at two different light intensities is presented in Table I.

The first column gives the f_m/f_0 ratio in State 2, the second column that in State 1. In the third and the fifth column of Table I the relative changes of f_m and f_0 caused by the State 2-State 1 transition are shown for two different light-2 intensities, demonstrating that at both light-2 intensities f_m increases more than f_0. At a light-2 intensity of 1 $W \cdot m^{-2}$ the relative changes of the f_m-level are twice as great as the corresponding f_0 changes as indicated by the sixth column. At a light-2 intensity of 10 $W \cdot m^{-2}$ the ratio between the relative changes is much closer to unity.

If state transitions are caused by changes of the absorption cross-section, the f_m/f_0-ratio should be the same in either state. The results of Table I and Fig. 2 show that this ratio does not remain constant. Deviations of this kind may be caused by a decrease in spillover or in energy quench in State 1. The energy quench which is caused by conformational changes of the thylakoid membrane as a consequence of a high proton concentration of the inner thylakoid space [10], can decrease the f_m-level of chlorophyll fluorescence up to 50%, whereas the f_0-level is scarcely influenced.

The possible involvement of energy quench in the results of Table I was studied by experiments, which made use of the fact that the energy quench vanishes about 30 s after switching off the light [9]. These experiments were performed at light-2 intensities of 1 $W \cdot m^{-2}$ and 10 $W \cdot m^{-2}$. The f_{m2}-level was determined as described above. Some minutes later, the f_m-level was determined a second time. In order to make the energy quench relax, all light sources were switched off for 35 s, before the measuring light and the saturating light were switched on simultaneously. The f_m-values determined by this procedure are labelled by an asterisk (f_{m2*}). In State 1, f_{m1} and f_{m1*} were determined by the same procedure. In the column 'q_E-test', the ratio $(f_{m1}/f_{m2})/(f_{m1*}/f_{m2*})$ is given, which is close to 1 at both light intensities. Thus, the f_{m*}-levels give the same ratio of f_m-enhancement. It has to be mentioned that during the dark period of 35 s also slight changes towards State 1 or State 2 might occur, but these changes are probably of minor influence because they occur on a much slower time-scale.

These control experiments provide strong evidence that the increase of the f_m/f_0-ratio in State 1 over that in State 2 is not caused by a decrease of energy quench. This finding is in line with the results of Weis and Berry [8] showing that in leaves adapted to light intensities below 20 $W \cdot m^{-2}$ the energy quench is of minor influence. However, as shown by Hansen et al. [11] even in this intensity range at least transient changes of the energy quench can occur. The lag phase in the f_m increase displayed in Fig. 1 might be explained by such a transient increase of the energy quench which is reversed during the following slow phase.

Since a significant contribution of energy quench can be ruled out by the above experiments, we assume that changes of the so-called spillover are involved. However, there are also changes of the absorption cross-section, as indicated by changes of f_0 of considerable magnitude.

The changes of the f_m/f_0 ratio at higher blue light intensities (10 $W \cdot m^{-2}$ and more) are relatively small, so that at these light intensities the changes of the absorption cross-section are dominating as already shown by Canaani and Malkin [6]. However, our data indicate that also at these light intensities spill-over changes do occur. The analysis of Malkin and coworkers [5], failed to detect changes in the f_m/f_0-ratio, probably because the experiments were performed at light intensities, where this ratio comes close to 1 (Fig. 2), and probably because these experiments made use of a too great intensity of the measuring light which led to an overestimation of f_0 as already mentioned by Bilger and Schreiber [12].

In-vitro studies (isolated chloroplasts) indicated mainly absorption changes at high salt conditions (Mg^{2+} concentration in the medium of 5 mM or greater), whereas at low salt conditions spill-over changes were found to dominate [2,3]. From measurements on intact plants indicating pure absorption changes (see above) it has been concluded that in-vivo the thylakoid membrane is always in a high salt state [4,13]. This conclusion does not seem to be valid any more in the light of our results. On the contrary, one might speculate that in-vivo light-dependent changes of the ionic en-

vironment of the thylakoid membrane determine the relative role of spillover in state transitions.

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