S14-003

Simultaneous measurement of photosystem I and photosystem II probed by modulated transmission at 820 nm and by chlorophyll a fluorescence in the sub ms to second time range.

RJ Strasser[1], G Schansker[1], A Srivastava[2], Govindjee[3]

[1]*Bioenergetics Laboratory, Geneva University, CH-1254 Jussy-Geneva, Switzerland (*Reto.Strasser@bioen.unige.ch*;* Gert.Schansker@bioen.unige.ch*; fax: 41-22-759-9945)*

[2]*Brown U., Providence, RI, 02912, USA (*Alaka_Srivastava@brown.edu*)*

[3]*Univ. of Illinois, Urbana, Ill, 61801-3707, USA (*gov@uiuc.edu*)*

Keywords: photosystem I, photosystem II, OJIP transient, P700, ΔA_{820}

Introduction

When light falls on photosystem I (PSI) and II (PSII) their antennae absorb light within femtoseconds; this is followed by excitation energy transfer to the reaction center chlorophylls (Chls) P700 (in PSI) and P680 (in PSII). Here the primary charge separation takes place leading to the production of $P700^+A_o^-$ and $P680^+Pheo^-$, where A_o is a bound Chl monomer and Pheo is a bound pheophytin molecule. $Pheo^-$ transfers electrons rapidly to a bound plastoquinone molecule Q_A^- (for details, see Ke 2001). In order to understand the interactions and the regulation of the two systems, it is necessary to measure simultaneous changes in the two systems. A large number of studies have dealt with the stoichiometry of PSI and PSII. Chl *a* fluorescence has been used since Duysens and Sweers (1963) as a monitor of Q_A^- and thus of PSII, and P700 since Kok and Hoch (1961) as a monitor of PSI. An early attempt to measure, at 77 K, P700 absorption and Chl *a* fluorescence simultaneously was made by Strasser and Butler (1976) who studied "spill over" of excitation energy from PSII to PSI. Schreiber *et al.* (1988) introduced parallel measurements for quantum yields of PSII (Chl *a* fluorescence) and PSI (absorbance change at 830 nm) in leaves by a modulated instrument. This method for simultaneous measurements of PSI and PSII was further improved by Havaux *et al*. (1991) and by Klughammer and Schreiber (1994, 1998), and recently exploited by Eichelmann and Laisk (2000) for the understanding of the cooperation between PSI and PSII in leaves. In this paper, we present simultaneous measurements of the 100 μs (for PSI) to 1 s range. P700 oxidation was measured by the relative light-induced absorption increase (ΔA_t) at 820 nm (decrease of the measured photocurrent I_{820}), whereas reduction of Q_A^- was measured by the variable Chl *a* fluorescence intensity (B_t). Our data provide new information on the relationship between the time-dependencies of PSI and PSII in the time range studied.

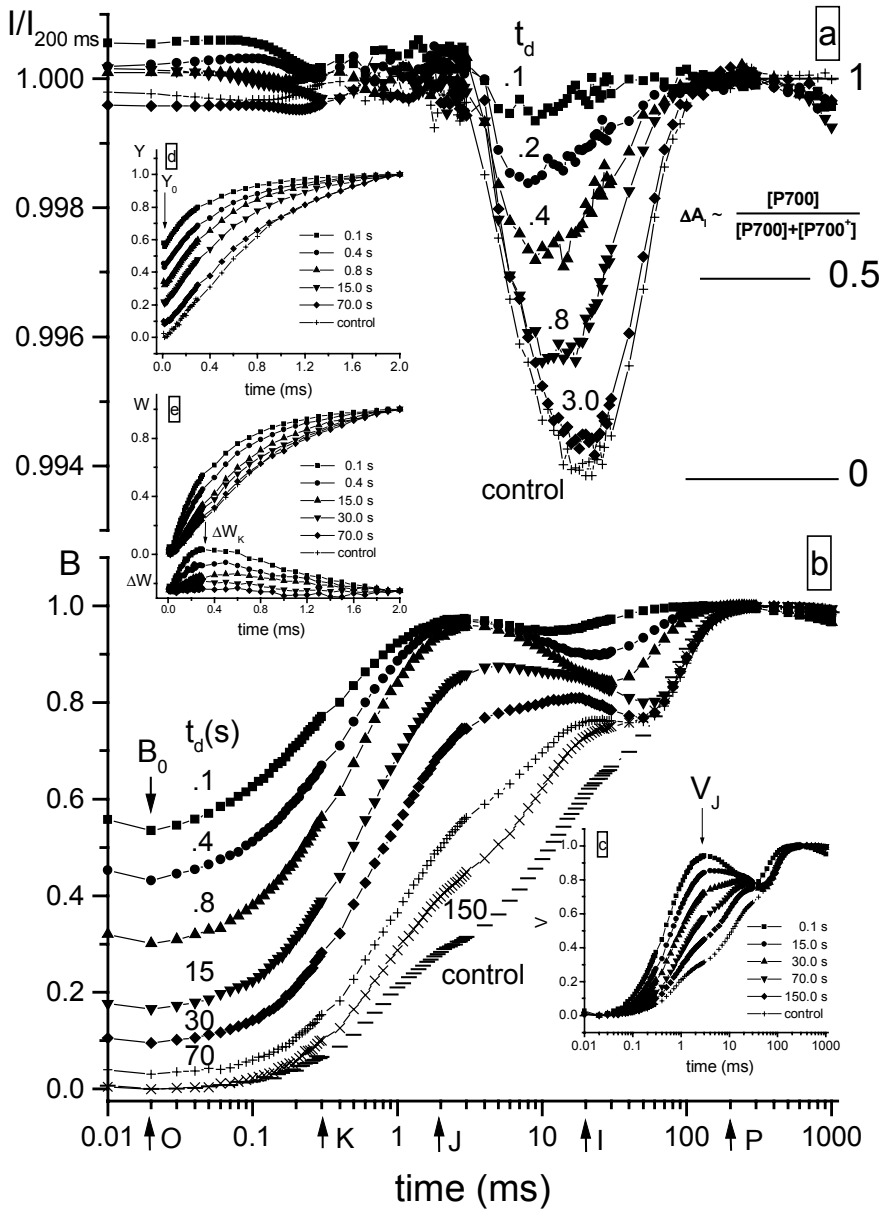

Fig. 1. Simultaneous measurements of the induction kinetics of PS II and PS I during a 1 s light pulse. Examples of induction kinetics (1 s) made at various times after the first pulse, as indicated in the figure, are plotted. (a.) Changes in photocurrent I at 820 nm, plotted as ΔI/I; (b.) OJIP-fluorescence induction curves, normalized at F_m (B_t) with $B_0 = (F_o'-F_o^d)/(F_m'-F_o^d)$; (c.) OJIP-curves double normalized to $F_{20\,\mu s}$ and F_m (V_t) with $V_J = (F_J'-F_o')/(F_m'-F_o')$; (d.) OJIP-curves normalized to F_J (Y_t) with $Y_0 = (F_o'-F_o^d)/(F_J'-F_o^d)$; (e.) OJIP-curves double normalized to $F_{20\,\mu s}$ and F_J (W_t). At the bottom of the graph the difference between the second and the first pulse is plotted as ΔW_t with $\Delta W_k = W_k'-W_k = (F_k'-F_o')/(F_J'-F_o')-(F_k^d-F_o^d)/(F_J-F_o)$. W_k and W_k' are calculated using for F_k and F_k', the average of the fluorescence values between 260 and 300 µs.
Abbreviations: F_o^d and F_m^d minimum and maximum fluorescence after 12 min dark adaptation (1st pulse); F_o', F_k', F_J' and F_m', fluorescence parameters derived from the second induction curve with the indices referring to the place on the OJIP-curve. measurements, on a logarithmic time scale, on $P700^+$ (PSI) and Q_A^- (PSII) changes in pea leaves exposed to continuous red (650 nm) light in the 10 µs (for PSII) and in the 100 µs to 1 s range (for PSI).

Table 1. Explanation of the symbols used in Fig. 1.

B	Fraction of closed reaction centers; normalisation of the transients to F_m and $F_{20\,\mu s}{}^d$ (d is dark-adapted leaf): $(F_t'-F_o^d)/(F_m'-F_o^d)$
V	Relative variable fluorescence; normalisation of the transients to F_m and $F_{20\,\mu s}$: $(F_t'-F_o')/(F_m'-F_o')$
Y	Scale used after normalisation of the transients to F_J and $F_{20\,\mu s}{}^d$ (single turnover range): $(F_t'-F_o^d)/(F_J'-F_o^d)$
W	Scale used after normalisation of the transients to F_J and $F_{20\,\mu s}$: $(F_t'-F_o')/(F_J'-F_o')$ $(= V_t/V_J)$
Subscripts:	
O, K, J, I	Fluorescence levels at respectively t = 20 µs, ~250 µs, 2 ms and 20 ms
P, M	Both maximum fluorescence level (around t = 200 ms)

Materials and methods

Measurements were made using Plant Efficiency Analysers (Hansatech Instruments, King's Lynn, Norfolk, England) combined with a high frequency modulated measuring beam at 820 nm. The instruments used were 2 HandyPEAs (Fig. 1) or 2 PEAs (Fig. 2) connected by a PEA 700 measuring system. For the measurements fully matured pea leave were used. The leaves were dark adapted for 12 minutes before the start of the measurement.

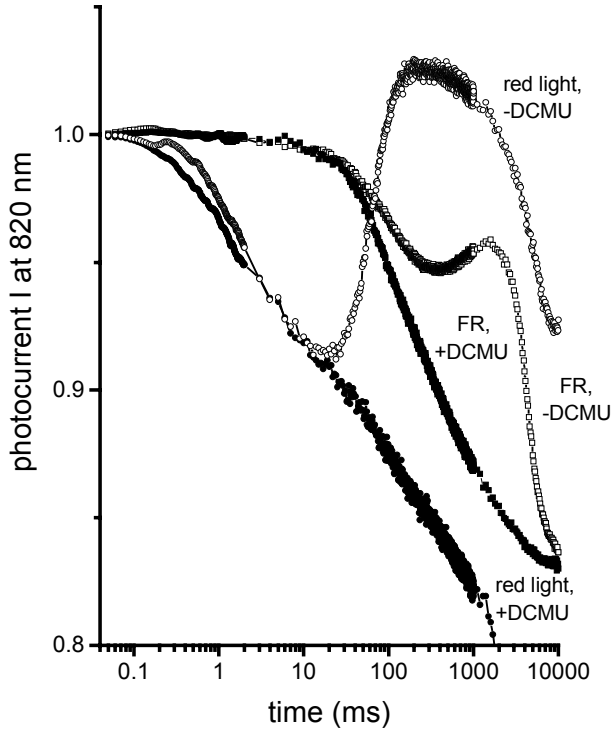

Fig. 2. Induction kinetics of P700 in the presence of combinations of red light, far-red light and DCMU. See text for details.

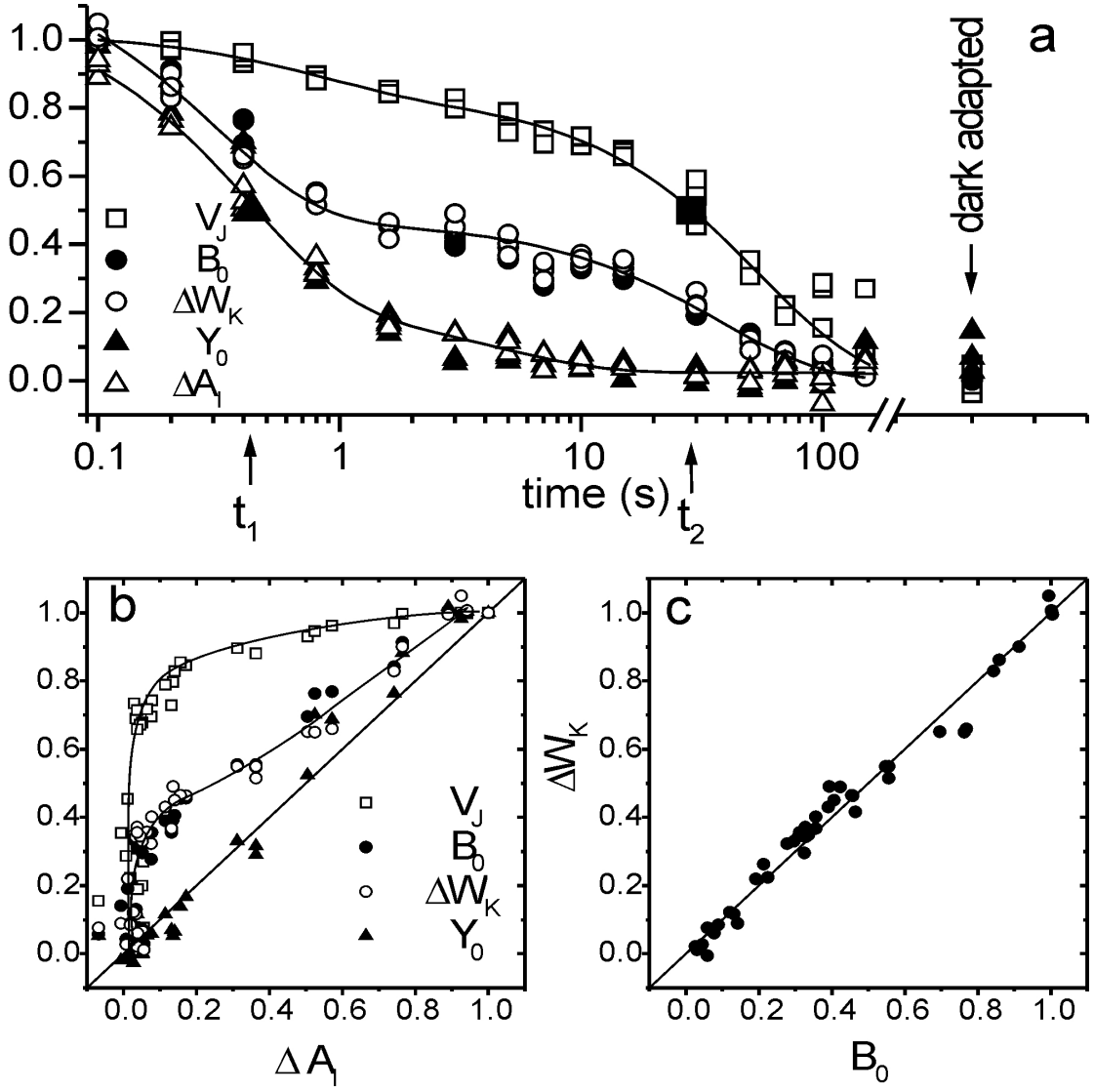

Fig. 3. The dark adaptation kinetics of V_J, B_0, ΔW_k, Y_0 (all for PSII) and ΔA_I (for PSI). The first 4 parameters were calculated as indicated in the legend of Fig. 1, and ΔA_I was calculated as $(1\text{-}I_{min}'/I_{200\ ms}')/(1\text{-}I_{min}/I_{200\ ms})$, where I_{min} is the average of several points at the lowest point of the transient around 20 ms (corresponding to the I-phase of the Chl a fluorescence transient). For details on Fig. 3b and 3c, see the text. In Figs. 3a and b trend lines were drawn through the symbols and in Figs. 3b and c the diagonals are indicated.

Simultaneous measurements of P700 and Chl a *fluorescence*

In Fig. 1 parallel measurements of the induction kinetics of both PSI and PSII in pea leaves during a 1 s light pulse of 3000 µmol m^{-2} s^{-1} are shown. Fig. 1a shows plots of the photocurrent I at 820 nm, whereas Fig. 1b shows the OJIP-kinetics of the fluorescence induction curves (Strasser *et al*. 1995 and 2000). A comparison of Figs. 1a and b shows that during the F_J-F_I-F_P rise (2-20-200 ms) the photocurrent I_{820} decreases and increases again, indicating an accumulation of $P700^+$ and a subsequent re-reduction to P700. The experiment illustrated in Fig. 1 was designed as a two pulse experiment in which the second pulse given at various times after the first was used to probe the dark adaptation kinetics of both signals. The insets c-e of Fig. 1 illustrate the various normalizations used in the paper; further Fig. 1

also introduces the parameters used later in Fig. 3. In Table 1, the various symbols used in Fig. 1 are defined.

In Fig. 2 the characteristics of the P700-transients, plotted in Fig. 1a, are further elaborated. In the presence of DCMU, the initial accumulation of $P700^+$ is not reversed but instead a further accumulation of $P700^+$ is observed. This is to be expected, since electron donation by PSII is blocked. The traces with and without DCMU initially run in parallel. The point at which they start to run apart should be at the moment electrons from PSII start to arrive in PSI, which is after about 20 ms. Far-red light that preferentially excites PSI is expected to have an effect comparable to DCMU. The trace shows that far-red light had an intermediate effect. A partial reversal of the initial accumulation of $P700^+$ is observed. Since, in the presence of DCMU, this partial reversal is completely eliminated this indicates that the far-red light also excited PSII to some extent. The lag-period observed in the traces with far-red light indicate that the excitation pressure of the far-red light was much lower than that of the red light used for the other two traces. Fig. 2 demonstrates that the PSI-feature plotted in Fig. 1a represents the balance between electrons leaving PSI and electrons coming in from PSII.

To understand the relationship between PSII and PSI better, 4 Chl fluorescence parameters: V_J, B_0, Y_0 and ΔW_k and the parameter ΔA_I (see Fig.1) were plotted as a function of the dark adaptation time (t_d) (Fig. 3a). The parameter V_J reflects the redox state of the intersystem electron transport chain (IETC). The idea is, that if the whole electron transport chain is reduced with the exception of Q_A, the maximum fluorescence will be reached within 2 ms (J-level). On the other hand, in a dark-adapted leaf many turnovers are needed to fully reduce the IETC and reach the F_m-level. As a consequence the J-level is much lower than the F_m. Fig. 1 shows that the parameters Y_0 and B_0 are expressions of the amount of variable fluorescence at the onset of the second illumination. In other words these two parameters are a function of the fraction of closed reaction centers at t = 0. Y_0 is normalized between F_o and F_J, which can be referred to as the single turnover range. This means that during this phase (0-2 ms) the redox state of the plastoquinone pool is hardly affected. B_0 is determined relatively to the F_m, which includes a full reduction of the IETC. Since it is known that the redox state of the plastoquinone pool modulates the fluorescence level (Vernotte *et al*. 1979) this may explain why the dark-adaptation kinetics of both parameters are not identical. The parameter ΔW_k reflects changes in the initial rate of photochemistry (see below).

Since the I_{820} transient seems to depend in part on the availability of electrons coming in from PSII one would expect a linear relationship between ΔA_I and the redox state of the IETC as reflected by V_J. As Fig. 3b demonstrates this is not the case. In contrast, a linear relationship between the ΔA_I and Y_0 (reflecting the redox state of Q_A) is observed. And if only the first phases of the dark adaptation kinetics of B_0 and ΔW_k are considered (Fig. 3a), these two parameters also seem to dark-adapt almost in parallel with ΔA_I. In other words the availability of intersystem electrons is in itself not enough to prevent the transient accumulation of $P700^+$.

When leaves are exposed to for example heat stress a new phase, designated the K-step, becomes visible in the OJIP-curve (Srivastava *et al*. 1997). It reaches a maximum at around 250 µs after the onset of light. Fig. 1d demonstrates that the difference between the induction curve of light-adapted leaves and dark-adapted leaves has a maximum around 250 µs. Heat destroys the donor side of PSII. The resulting faster induction phase has been interpreted to indicate that the few electrons that can still be donated to Tyr_Z under these conditions can be donated at a faster rate without the kinetic constraints of the oxygen-evolving complex. The result is a steeper initial slope. With the parameter ΔW_k we try to probe the properties of the K-step in unstressed plants. Since the value W_k is so closely related to the initial slope of the fluorescence induction curve it is a practical measure for the normalized slope of the fluorescence rise at the origin: $(dV/dt_0)/V_J = dW/dt_0 \sim W_k$. This expression corresponds

according to the JIP-test to the flux of excitons trapped per time and per reaction center (TR_0/RC) at the onset of the second illumination (Strasser *et al.*, 2000). Figs. 3a and c show that there is a linear relationship between the parameter B_0 and ΔW_K. A ΔW_k larger than 0, which is observed at short times after the first pulse, indicates an increase of this trapping flux. In other words, electron donation to Tyr_Z seems to occur at a faster rate after a pre-illumination.

An increase in the donation rate on the donor side of PSII can be explained in several ways. In the past there has been a considerable amount of speculation about the existence of cyclic electron transport around PSII. The correlation between the redox state of Q_A and the donation rate on the donor side can then be explained by a certain probability that an electron on Q_A will flow back to Tyr_Z via an unknown pathway increasing the number of electrons that can be donated to Tyr_Z. Alternatively, we can use the lumen pH as an explanation. In dark-adapted leaves the lumen pH will be around 7.5, which is sub-optimal for the oxygen-evolving complex. In the first seconds after the end of the first pulse the lumen pH is still low allowing the oxygen-evolving complex to turn over faster than in dark-adapted leaves. This would explain the increase of the donation rate, but not the correlation with the redox state of Q_A.

Acknowledgements

This work was supported by Swiss National Foundation grant no. 3100-057046.99/1.

References

Duysens, L.N.M. and H.E. Sweers (1963) In: Studies on Photosynthetic Bacteria and Microalgae (Japanese Society of Plant Physiologists, eds.), pp. 353-372. University of Tokyo Press, Tokyo.

Eichelmann, H. and A. Laisk (2000) *Plant Cell Physiol*. **41**: 138-147.

Havaux, M., H. Greppin and R.J. Strasser (1991) *Planta* **186**: 88-98.

Ke, B. (2001) Photosynthesis: Photobiochemistry and Photobiophysics, pp. 763. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Klughammer, C. and U. Schreiber (1994) *Planta* **192**: 261-268

Klughammer, C. and U. Schreiber (1998) In: Photosynthesis: Mechanisms and Effects (G. Garab, ed.), **V**: 4357-4360.

Kok, B. and G. Hoch (1961) In: Light and Life (W.D. McElroy and B. Glass, eds.), pp. 397-416. Johns Hopkins, Baltimore, MD.

Schreiber, U., C. Klughammer and C. Neubauer (1988) *Z. Naturforsch*. **43c**: 686-698.

Srivastava, A., B. Guissé, H. Greppin and R.J. Strasser (1997) *Biochim Biophys Acta* **1320**: 95-106.

Strasser, R.J. and W.L. Butler (1976) *Biochim Biophys Acta* **449**: 412-419.

Strasser, R.J., A. Srivastava and Govindjee (1995) *Photochem. Photobiol*. **61**: 32-42.

Strasser, R.J., A. Srivastava, A. and M. Tsimilli-Michael (2000) In: Probing Photosynthesis (Yunus, M., U. Pathre and P. Mohanty eds.), pp. 445-483. Taylor and Francis, London.

Vernotte, C., A.L. Etienne and J.-M. Briantais (1979) Biochim Biophys Acta 545: 519-527.