RAPID LIGHT-INDUCED CHANGES OF ENERGY DISTRIBUTION BETWEEN PHOTOSYSTEMS I AND II

Ulrich SCHREIBER and William VIDAVER

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

Received 24 November 1975

1. Introduction

Chlorophyll fluorescence induction phenomena (Kautsky effect) have been very useful in the study of photosynthesis (for a review see [1]. It has long been assumed that the fluorescence induction kinetics are independent of measuring wavelength. Recently it was discovered in Butler's laboratory that the F690/F730 ratio undergoes changes during the slower part of the induction period (R. J. Strasser, personal communication), where other investigators [2–4] had already observed fluorescence changes, independent of photosystem II acceptor Q*. These slow fluorescence changes are believed to reflect ion-induced changes in energy distribution between the two photosystems (for reviews see [1,5,6]). Any increase in the energy fraction distributed to System I should lead to an increase of System I fluorescence centered around 735 nm relative to System II fluorescence at 685 nm.

In earlier work we concluded that not only slow fluorescence changes but also certain rapid transients are due to changes of energy 'spill-over' from pigment system II to pigment system I [7,8]. If this conclusion is correct, rapid changes in the ratio of System I and System II fluorescence are also expected. With a system developed to monitor fluorescence ratios automatically and having a time resolution under 1 ms, we did discover very fast changes. Our new data support earlier conclusions of rapid energy distribution changes and suggest a 'switch' mechanism, which operates far faster than ion-induced conformational changes of the thylakoid membrane could occur.

Abbreviations: DCMU, 3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea; Q, primary electron acceptor of photosystem II.

2. Materials and methods

All experiments were with *Phaseolus vulgaris* at room temperature (≈22°C). Fluorescence excitation was by a broad blue band from a halogen lamp together with Corning 9782 and Balzers K6 (without cut-off filter) filters. Illumination and collection of surface fluorescence was achieved by trifurcated fiberoptics [9]. Photomultipliers (EMI 9658 R) were screened by Balzers 691 and 750 nm B-20, 8 nm half-width filters. Due to the spectral sensitivity of the phototubes and in particular the non-perpendicular exit angle of fluorescence from the fibres, peak sensitivities were at 685 and 735 nm. Photomultiplier anode currents were fed into operational amplifiers; dark currents were compensated. By applying an appropriate voltage to the amplifier non-inverting input the constant fraction of the 735 nm initial signal ['α-fraction'] was compensated as well. The ratio of the two signals was recorded automatically by use of an analog divider (Burr and Brown, model 4290). Response time was limited by RC values at the amplifiers and was approx. 500 μs. Ratio curves, individual fluorescence curves, and X-y plots of F 735 versus F 685 were recorded on a dual beam storage oscilloscope (Tektronix, 5103 N) and photographed.

3. Results and discussion

It is known for some time that the ratio of initial fluorescence (F_0) to variable fluorescence (F_v) increases in the long wavelength emission region [10]. In Butler's latest model [11] F_0 735 is composed of two fractions, one due to direct absorption by System I

North-Holland Publishing Company – Amsterdam

pigments [α-fraction] and the other arising from energy transfer from System II pigments. The α-fraction is constant throughout illumination unless membrane conformational changes alter the cross-section of absorption. F_v 735 is exclusively from energy transfer by System II pigments and governed by the same laws as F_v 685 as long as energy distribution changes are prevented (e.g. at −196°C) [11]. Even at room temperature it is correct to assume

$$\frac{d\,685}{d\,735} = \frac{F_0\,685}{F_0\,735 - \alpha\text{-fraction}}, \quad \text{if} \quad \frac{d\,685}{d\,735}$$

is measured immediately after onset of illumination. This can be done by rapid plotting of 735 versus 685 nm on a storage oscilloscope. The intercept of the initial slope at the 735-axis then gives the α-fraction. In bean leaves and with the particular excitation band applied in our experiments this term was approx. 1/3 F_0 735. In order to collect information concerning changes in energy distribution we have compensated for the α-fraction, as this appears not to be under the immediate control of energy transfer parameters.

Fig.1a shows light-induced transients of F 685/F 735 in a bean leaf. Very similar ratio curves, not shown here, have been measured in a variety of plants. The maximum ratio change observed is on the order of 20%. All ratio curves display a rapid initial spike P_1 (after approx. 25 ms at the given conditions) followed by a slower peak P_2 (after approx. 250 ms). Slower recordings show at least two additional peaks at approx. 2.5 s and 25 s. We refrain from speculating on the significance of this decade aspect, although it may be too regular to be coincidental. In fig. 1b the corresponding F 685 and F 735 curves are shown. Even by visual comparison the difference in the two induction curves is obvious, although much more so in the region of P_2 than of P_1. The ratio curves are sensitive to all factors affecting photosynthesis, as are the individual fluorescence curves. We show only the effect of saturating DCMU (fig.2) which is presumed to block electron transport completely. Fig.2a gives ratio curves at two recording rates; fig.2b shows the corresponding traces for the individual emissions. In the ratio curves there is an extremely rapid rise followed by a slower decay. The speed of this transient can be appreciated from the fact that its peak occurs after only approx. 1/3 to 1/2 of the DCMU fluorescence rise is completed. We found, that as with the individual emission curves the ratio curves obey the I × t law. With our highest light intensities at the present slew rate of our ratio amplifier, half-rise times of <500 μs were evident.

We considered various possibilities of artifacts affecting the ratio transients. Electronic artifacts are ruled out, as point by point calculation of ratios from the individual emission curves were identical to recorded curves. Errors in compensation of the α-fraction change the shape of the ratio transients in the region of lower

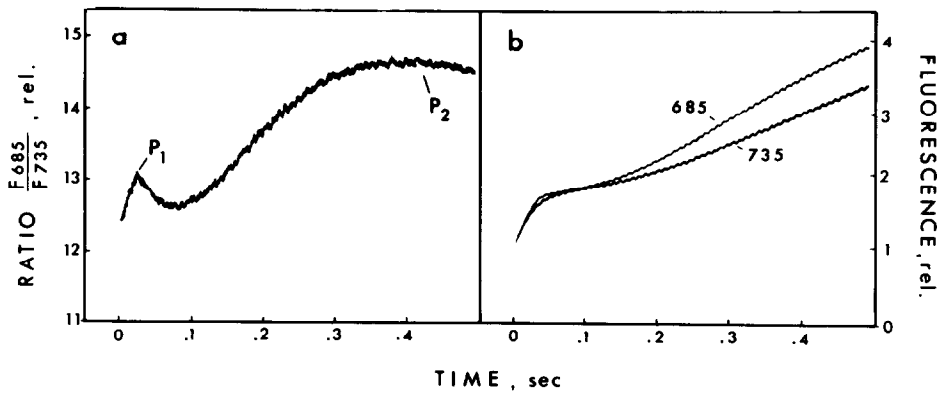

Fig.1 (a) Fluorescence ratio curve F 685/F 735 as compared to (b) the individual fluorescence curves. All curves recorded from different areas of the same dark adapted bean leaf. Light intensity 2 mW cm^{-2}. In (a) the 735 nm signal was compensated continuously for 0.34 × F_0 735. In (b) the 735 nm curve was compensated by the same value, and the two curves were matched at beginnings and ends by adjusting sensitivities. Note: The two curves in (b) do not cross. The ordinate in (a) gives the recorded ratio as voltage output from the amplifier and bears no numerical significance concerning the actual emission ratio.

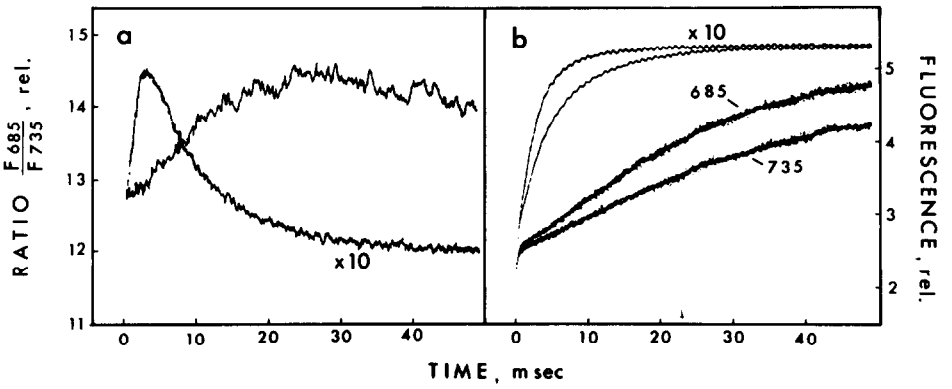

Fig.2 Inhibition by DCMU, (a) F685/F735 ratio transients at two different rates of recording; α-fraction compensation was equivalent to 0.28 × $F_0$735. (b) Individual fluorescence curves recorded under the same conditions as (a). Curves were matched at beginnings and ends by adjusting sensitivity. Light intensity, 0.4 mW cm^{-2}. DCMU concentration was saturating. Bean leaf discs were soaked in the inhibitor solution for 8 h in the dark before experiments. Note: The curves marked by × 10 were recorded at 10 × the rate given on the abscissa. For ordinate, see legend to fig.1.

fluorescence values, but rapid transients occur for any compensation value. Changes in fluorescence reabsorption can be expected in whole leaves with induced structural changes of the chloroplasts. But we found typical ratio transients in isolated chloroplasts and furthermore the early transients are far too fast to reflect membrane conformational changes.

While slow ratio changes are predicted by the work of others [2–4, 12–17], we believe we have observed the first experimental indication of a rapid type of energy distribution control, such as proposed by Seely [18] in a computer model. The DCMU experiment suggests that only the rate of quantum absorption limits the speed of the effect. We believe that light induced ion fluxes, except possibly proton fluxes, can be ruled out as control modulators for the fast changes. More probable is control via the electric field across the thylakoid membrane, which is built up simultaneously with charge separation at the reaction centers [19].

The above data also bear some importance for a number of other aspects of photosynthesis research. The essence of our findings appears to be that there is an immediate change in energy distribution following a sufficiently high light stimulus. This may explain certain periodic phenomena following flash excitation in oxygen exchange and fluorescence (for a review see [20]). In this respect it is important to note that after suitable pre-illumination rapid O_2-uptake spikes are observed, showing a System I action spectrum [21].

Our new data suggest that the long prevailing idea that rapid fluorescence transients are independent of energy distribution changes has to be corrected, as one of us proposed earlier [7,8]. As it now appears charge separation at the reaction centers results in a brief stimulation of System II excitation causing a rapid O-I rise for two reasons, (a) because of the higher System II excitation, and (b) because of rapid reduction of Q. At low light intensities there is still an O-I rise to the so-called F_p-level [22], which as Q stays oxidised reflects only the effect of (a). The switch to favored System I excitation comes quite abruptly, as can be seen from the shape of P_1 in fig.1a. This will immediately cause quenching of variable fluorescence and only after a delay will there be additional quenching due to increased Q-reoxidation via the electron transport chain. In this sense the whole of fluorescence induction reflects a composite of direct energy transfer changes and indirect redox changes in Q.

Acknowledgements

We wish to thank Dr R. Strasser who revived our interest in 'spill-over'. We gratefully acknowledge support from the Simon Fraser University Faculty of Science and the Department of Biology, and support by National Research Council of Canada operating grant A2908.

References

[1] Govindjee and Papageorgiou, G. (1971) in: Photophysiology (Giese, A. C., ed.) Vol. 6, pp. 1–46, Academic Press, New York.
[2] Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251.
[3] Bonaventura, C. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366–383.
[4] Wraight, C. A. and Crofts, A. R. (1970) Eur. J. Biochem. 17, 319–327.
[5] Hind, G. and McCarty, R. E. (1973) in: Photophysiology Giese, A. C., ed.) Vol. 8, pp. 113–156, Academic Press, New York.
[6] Papageorgiou, G. (1975) in: Bioenergetics of Photosynthesis (Govindjee, ed.) pp. 320–371, Academic Press, New York.
[7] Schreiber, U. (1968) Diplomarbeit, RWTH Aachen, Germany.
[8] Schreiber, U., Bauer, R. and Franck, U. F. (1971) in: Proc. 2nd Int. Congr. Photosynthesis Res., Stresa (Forti, G., Avron, M. and Melandri, A. eds.) Vol. 1, pp. 169–179.
[9] Schreiber, U. and Vidaver, W. (1973) Z. Naturforsch. 28c, 704–709.
[10] Lavorel, J. (1962) Biochim. Biophys. Acta 60, 510–523.
[11] Butler, W. L. and Kitajima, M. (1975) Biochim. Biophys. Acta 396, 72–85.
[12] Duysens, L. N. M. and Sweers, H. E. (1963) in: Studies on Microalgae and Photosynthetic Bacteria (Miyachi, S., ed.), Special Issue of Plant Cell Physiol., Tokyo, pp. 353–372.
[13] Murata, N. (1971) Biochim. Biophys. Acta 226, 422–432.
[14] De Kouchskovsky, Y. (1971) in: Proc. 2nd Int. Congr. Photosynthesis Res., Stresa (Forti, G., Avron, M. and Melandri, A., eds.) Vol. 1, pp. 233–245.
[15] Mohanty, P., Zilinskas Braun, B. and Govindjee (1973) Biochim. Biophys. Acta 292, 459–476.
[16] Briantais, J. M., Vernotte, C. and Moya, I. (1973) Biochim. Biophys. Acta 325, 530–538.
[17] Gross, E. L. and Hess, S. C. (1973) Arch. Biochem. Biophys. 159, 832–836.
[18] Seely, G. R. (1973) J. Theor. Biol. 40, 188–198.
[19] Witt, H. T. (1971) Q. Rev. Biophys. 4, 365–477.
[20] Joliot, P. and Kok, B. (1975) in: Bioenergetics of Photosynthesis (Govindjee, ed.) pp. 387–412, Academic Press, New York.
[21] Vidaver, W. and French, C. S. (1965) Plant Physiol. 40, 7–12.
[22] Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) Photochem. Photobiol. 14, 287–305.