ATP-DEPENDENT STATE 1–STATE 2 CHANGES IN ISOLATED PEA THYLAKOIDS

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Received 2 May 1981

1. Introduction

Bonaventura and Myers [1] introduced the concept of state 1–state 2 changes to describe the physiological process by which photosynthetic oxygen evolving organisms can regulate the distribution of absorbed light energy between photosystem I and II (PSI and PSII) so as to maintain a maximum quantum efficiency for non-cyclic electron transport. These changes are readily observed in algae [2–8] and more recently have been detected in the leaves of higher plants [9,10]. The mechanism of the control process almost certainly involves changes in spillover of energy between the light harvesting chlorophylls of PSII and PSI as demonstrated by chlorophyll fluorescence yield changes at room temperature and at 77 K [8]. The resulting changes in quantal efficiency can be monitored either by measuring O_2 evolution or by monitoring the steady-state level of chlorophyll fluorescence under conditions when the emission yield can be closely correlated with the reduction level of the primary electron acceptor of PSII, often called Q [1–8]. State 1 occurs when there is an excess of PSI light (light 1) so that spillover is at a minimum while state 2 is the maximum spillover condition associated with excess PSII light (light 2).

Light-dependent phosphorylation of the PSII light harvesting chlorophyll *a/b* protein (LHCP) has been demonstrated in [11,12]. This process is accompanied by an increase in spillover of excitation from PSII to PSI and the kinase reaction involved appears to be regulated by the redox state of the plastoquinone pool [13,14]. Therefore it seems that the shift from state 1–state 2 may be due to the phosphorylation process induced when PSII is over excited [15].

Here, we demonstrate reversible ATP dependent state 1–state 2 changes in isolated pea thylakoids brought about by kinase/phosphatase activity and show that they are accompanied by changes in spillover from PSII to PSI.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; LHCP, Light-harvesting chlorophyll *a/b* protein complex; PS, photosystem; Q, primary acceptor of photosystem II

2. Materials and methods

Changes in chlorophyll fluorescence yield of pea leaves and envelope free pea thylakoids were measured using a modulated blue-green light (light 2) of irradiance 1.3 W/m^2 transmitted by Schott BG18 (4 mm) and BG38 (2 mm) filters. The modulated emission at 686 ± 11 nm was detected by a photomultiplier connected to a lock-in amplifier. When required, non-modulated light 1 at 710 ± 12 nm (transmitted through a Balzer interference filter) was introduced at an intensity of ~7.5 W/m^2. The set-up involved the use of a 3 branched optical fibre system as in [9]. The optic fibre mixing tube was either held vertically over the surface of a pea leaf or horizontally against the side of a 10 × 10 × 45 mm glass cuvette containing pea thylakoids which was stirred continuously from below. Liquid N_2 temperature emission spectra were measured using a Perkin-Elmer MPF 44A spectrofluorimeter as in [16]. Samples in cylindrical quartz tubes bathed in liquid N_2 were excited with 435 nm light (slit width 10 nm) and emission spectra were obtained with a slit width of 5 nm.

Intact chloroplasts were isolated from pea leaves as in [17] except that they were finally resuspended in reaction medium (see later) plus 3 mM $MgCl_2$ and kept on ice as a concentrated stock. To investigate

the involvement of protein phosphorylation in state 1–state 2 changes, aliquots from the stock were osmotically shocked in the presence of 6 mM $MgCl_2$ at 4°C for 60 s. Double-strength reaction medium was then added to bring the final concentration of constituents to 15 μg chl/ml, 0.33 M sorbitol, 4 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 50 mM Hepes, pH adjusted to 7.6 with KOH. 6 μM ferredoxin, 0.5 μM nigericin and 0.5 μM valinomycin were also added. Chlorophyll (chl) concentration was determined as in [18]. *Spirulina maxima* ferredoxin was the generous gift of Dr K. K. Rao, King's College, London.

3. Results

Fig.1a shows the change from state 1–state 2 in an intact pea leaf attached to the plant due to illumination with light 2. Prior to the measurement, the leaf had been adapted to state 1 by illumination with light 2 plus excess light 1 for 20 min. On turning off light 1 the imbalance in the excitation of the 2 photosystems is demonstrated by the immediate increase in fluorescence yield due to the further reduction of Q by the excess PSII light. This increase is followed by a slow decrease ($t_{1/2} \sim 1.25$ min) in the fluorescence yield which may be attributed to 3 effects:

(i) Increase in spillover from PSII to PSI;
(ii) Oxidation of reduced Q due to the increased quantal efficiency of PSI relative to PSII and hence to spillover;
(iii) High energy state quenching [8].

The change in quantal efficiency of PSI relative to PSII can be monitored, as shown in fig.1b, by superimposing light 1 for short periods at various times during the fluorescence quenching. As fig.1a shows, when the leaf is fully adapted to state 2, the effect of introducing light 1 is very small indicating that under the particular illumination used, the 2 photosystems have become more-or-less balanced. The increase in fluorescence yield when light 1 is turned off is ~70% of the total fluorescence yield observed in light 1 plus light 2. This indicates that Q is only partially reduced since the increase would be expected to be greater than this and is a consequence of working at limiting light intensities (see below the effect of DCMU on fluorescence from isolated chloroplasts).

Fig.2 shows the effect of light 1 and 2 on the chlorophyll fluorescence yield of isolated pea thylakoids

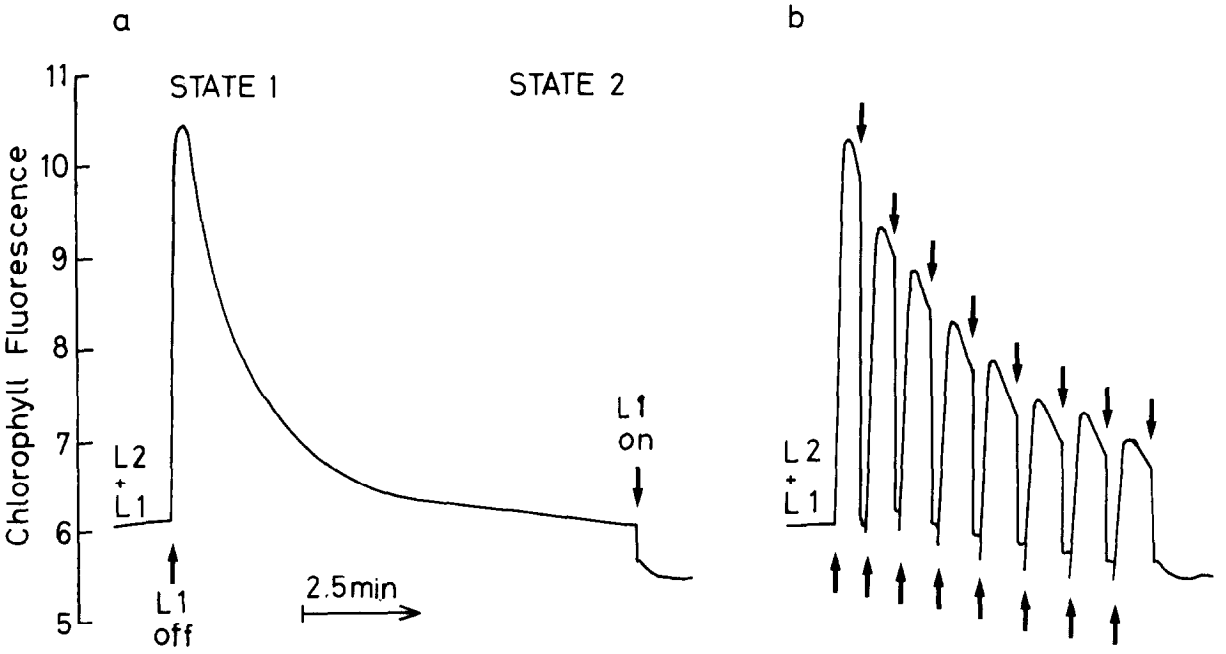

Fig.1. Relative changes in the yield of modulated chlorophyll fluorescence from pea leaves attached to the plant. In each case the leaf was preilluminated with light 2 plus light 1 for 20 min. (a) Kinetics of the fluorescence transient observed after turning off light 1 (state 1–state 2). (b) Effect of brief periods of illumination with light 1 during the state 1–state 2 change. Upward and downward arrows indicate light off and on, respectively.

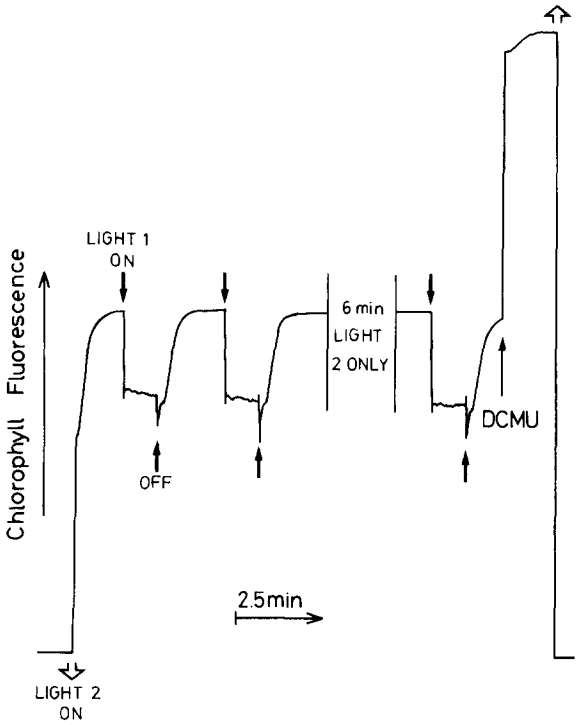

Fig.2. Effect of superimposition of light 1 on the relative yield of modulated chlorophyll fluorescence excited by light 2 from isolated pea thylakoids incubated with ferredoxin and uncoupler. DCMU (20 μM) was added where indicated. Experiment carried out at room temperature.

in the presence of 3 mM $MgCl_2$ (note 2 mM divalent metal chloride is chelated by EDTA). The membranes were supplied with ferredoxin as an electron acceptor and were uncoupled with a combination of nigericin and valinomycin. The presence of 3 mM $MgCl_2$ places these chloroplast membranes in state 1, i.e., spillover is at a minimum [19]. Under these conditions, as expected, the effect of superimposing light 1 on light 2 is to oxidise reduced Q and bring about a lowering of fluorescence. However, unlike the intact leaf, no adaptation to state 2 occurred on continuous illumination with light 2 only. Addition of DCMU increased the fluorescence yield due to the complete reduction of Q indicating that light 2 alone at the intensity used and in the presence of ferredoxin was not able to reduce the primary PSII acceptor fully.

The results in fig.2 contrast with those in fig.3 which shows the effect of supplying the thylakoids

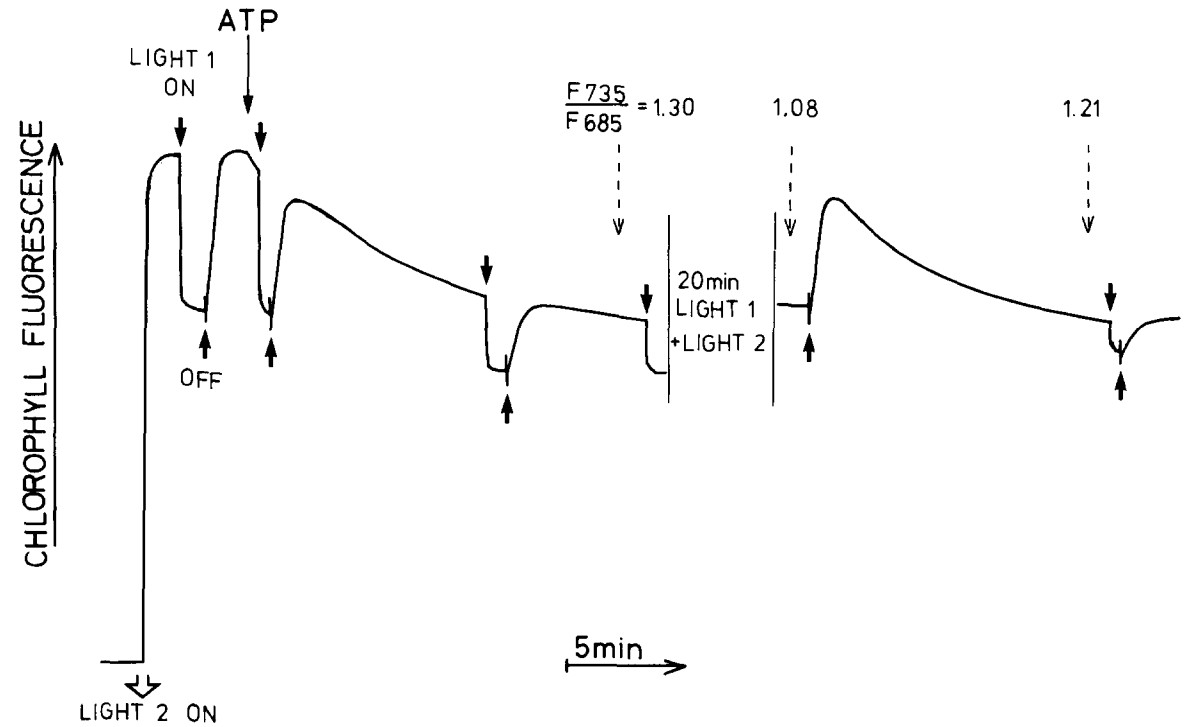

Fig.3. Induction of state 1–state 2 changes in isolated pea thylakoids by the addition of ATP (0.2 mM). Where indicated (——→) duplicate samples were taken for low temperature fluorescence emission spectroscopy. The average F735/F685 ratios obtained are noted on the figure.

with ATP as well as ferredoxin and uncoupler. Light 1 was turned on briefly to show the degree of imbalance of quantal distribution of light 2 between the 2 photosystems before adding ATP. After addition of 0.2 mM ATP there was a steady decrease in the fluorescence yield ($t_{1/2} \sim 4$ min) which correlated with a decrease in the ability of light 1 to oxidise reduced Q. Under these conditions the isolated thylakoids appear to behave qualitatively like the intact pea leaf and indicate that in the presence of ATP, light 2 was able to bring about a change from state 1–state 2. As shown in fig.3, after adaption to state 2 the isolated thylakoids were then illuminated with light 1 plus light 2 for 20 min which brought about a slow increase in fluorescence yield approximately to the level seen before adaptation to state 2 in the presence of light 1 plus light 2 (i.e., state 1 level). When light 1 was turned off, the fluorescence increased to a peak and then slowly requenched to the state 2 level. The dependence on ATP and reversibility in the dark of these transitions between state 1–state 2 suggest that the process is controlled by the phosphorylation of the LHCP [13,14].

In order to test whether these changes in state 1–state 2 were accompanied by changes in spillover between PSII and PSI, samples were taken at the times indicated in fig.3 for low temperature fluorescence spectroscopy. The F735/F685 ratios for the 3 samples are given in fig.3. After illumination with light 2 (state 2) the ratio was 1.30, then after illumination with light 2 plus excess light 1 (state 1) the ratio was decreased indicative of an increase of PSII fluorescence relative to PSI due to decreased spillover. A second adaptation with light 2 increased the ratio again towards the higher maximum spillover value. In this experiment the samples were illuminated for ~15 s at room temperature and during freezing with bright white light in order to fully reduce Q. In another experiment, DCMU was added just prior to freezing and the F735/F685 ratios for plus and minus ATP conditions, were measured. The values plus and minus ATP were 1.84 and 1.41, respectively, corresponding to a 30% relative increase brought about by ATP which is smaller than the 55% increase observed for intact pea leaves adapted to state 2 and state 1 [9].

4. Discussion

This work indicates that isolated thylakoids are capable of showing changes in quantal distribution associated with state1–state 2 adaptation which, with one exception [19], had been demonstrated only in intact tissue. This regulatory phenomenon is dependent on the spectral quality of the incident radiation and on the presence of ATP, and independent of the high energy state since it occurs in the presence of uncouplers. The results strongly support the argument that the phosphorylation/dephosphorylation of the exposed segment of the LHPC complex at the membrane surface controls excitation transfer between the antenna chlorophylls of PSII and PSI [12–15]. Moreover, the antagonistic effect of light 1 and 2 is consistent with the concept that it is the redox level of plastoquinone which controls the kinase/phosphatase activity [13,14]. The role of the phosphorylation/dephosphorylation processes could be to change the surface electrical properties of the PSII LHCP complex in such a way as to alter its Coulombic interaction with the light-harvesting chlorophyll protein complex of PSI and thus bring about spatial changes which increase or decrease energy transfer from chlorophyll beds of the adjacent pigment complexes [20].

Acknowledgements

The work has been possible because of financial support from the Science Research Council and Agricultural Research Council.

References

[1] Bonaventura, C. J. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366–383.
[2] Duysens, L. N. M. and Talens, A. (1969) in: Progress in Photosynthesis Research (Metzner, H. ed) vol. 2, pp. 1073–1087, Univ. Tubingen, Tubingen.
[3] Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251.
[4] Bennoun, P. and Jupin, H. (1974) in: Proc. 3rd Int. Cong. Photosynthesis (Avron, M. ed) vol. 1, pp. 163–169, Elsevier/North-Holland, Amsterdam, New York.
[5] Vernotte, C., Briantais, J. M., Armond, P. and Arntzen, C. J. (1975) Plant Sci. Lett. 4, 115–123.
[6] Williams, W. P., Furtado, D. and Nutbeam, A. R. (1980) Photobiochem. Photobiophys. 1, 91–102.
[7] Ried, A. and Reinhardt, B. (1980) Biochim. Biophys. Acta 592, 76–86.
[8] Barber, J. (1976) in: The Intact Chloroplast (Barber, J. ed) Top. Photosynth., vol. 1, pp. 89–134, Elsevier/North-Holland, Amsterdam, New York.

[9] Chow, W. S., Telfer, A., Chapman, D. J. and Barber, J. (1981) Biochim. Biophys. Acta submitted.
[10] Baker, N. R. (1981) in: Plants and the Daylight Spectrum (Smith, H. ed) Academic Press, London in press.
[11] Bennett, J. (1977) Nature 269, 344–346.
[12] Bennett, J. (1979) FEBS Lett. 103, 342–344.
[13] Bennett, J., Steinback, K. E. and Arntzen, C. J. (1980) Proc. Natl. Acad. Sci. USA 77, 5253–5257.
[14] Horton, P. and Black, M. T. (1981) Biochim. Biophys. Acta 635, 53–62.
[15] Bennett, J. (1979) Trends Biochem. Sci. 4, 268–271.
[16] Telfer, A., Nicolson, J. and Barber, J. (1976) FEBS Lett. 65, 77–83.
[17] Nakatani, H. Y. and Barber, J. (1977) Biochim. Biophys. Acta 461, 510–512.
[18] Arnon, D. I. (1949) Plant Physiol. 24, 1–15.
[19] Horton, P. and Black, M. T. (1980) FEBS Lett. 119, 141–144.
[20] Barber, J. (1980) FEBS Lett. 118, 1–10.