

The relationship between changes in the redox state of plastoquinone and control of excitation energy distribution in photosynthesis

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The hypothesis that excitation energy distribution between PS I and PS II is controlled by the redox state of the plastoquinone pool between the two photosystems was investigated using the green alga *Chlorella vulgaris*. Changes in the redox state of the pool were monitored by measurement of the area above the fluorescence induction curve on exposure to high-intensity light. In agreement with the hypothesis, exposure of state I adapted cells to light preferentially absorbed by PS II led to a reduction of the plastoquinone pool whilst exposure of State II adapted cells to light preferentially absorbed by PS I resulted in its oxidation. However, the limits within which these fluctuations occurred were much narrower than anticipated. The reasons for this are discussed in terms of the possible involvement of changes in the redox state of more specialised molecules associated with the main plastoquinone pool and the postulated role of plastoquinone as an electron shuttle between the two photosystems.

Photosynthesis Plastoquinone State I State II

1. INTRODUCTION

The distribution of excitation energy between photosystem I (PS I) and photosystem II (PS II) in algal cells [1,2] and higher plants [3] is determined by the wavelength of the incident light. When light is preferentially absorbed by PS I, the cells undergo a transition to a state (State I) characterised by a higher efficiency of photoprocesses associated with PS II whilst preferential absorption of light by PS II leads to a state (State II) characterised by a higher efficiency of PS I photoprocesses. These changes are thought to be controlled by changes in the interaction of the light-harvesting chlorophyll *a/b*-protein complex (LHCP) with the two photosystems [4]. LHCP is phosphorylated by a kinase system that is activated when the incident light is predominantly absorbed by PS II [5]. Phosphorylation of LHCP is believed

to lead to a migration of LHCP from the PS II-rich appressed membranes of the chloroplast into PS I-rich non-appressed membrane regions [6], or to a migration of PS I light-harvesting complexes from the non-appressed to appressed regions [7], that results in an effective increase in the absorption cross-section of PS I with respect to PS II.

The initial detection of any imbalance between the amounts of excitation energy reaching the two photosystems is thought to be sensed via the redox state of the plastoquinone (PQ) pool located between PS II and PS I [5,8]. Horton et al. [8] have demonstrated that the extent of LHCP phosphorylation by exogenous ATP in redox-poised chloroplasts is directly related to the redox state of the PQ pool. Under conditions where PQ is reduced, LHCP is phosphorylated and when PQ is oxidised LHCP remains non-phosphorylated. Here we have investigated the changes in the redox state of the PQ pool of intact *Chlorella* cells as they adapt to State I and State II and show that it

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is likely to be the redox state of molecules closely associated with the PQ pool, rather than that of the pool itself, that is of importance in the control of excitation energy distribution.

2. MATERIALS AND METHODS

Chlorella vulgaris (211/8k) cells were obtained from the ITE Culture Centre (Cambridge) and grown under continuous culture conditions [9]. The redox state of the PQ pool of the cells was estimated from fluorescence induction measurements, by the method of Malkin and Kok [10,11], using the apparatus shown in fig.1. Aliquots of cells harvested in their log-phase, were placed in the thermostatted cuvette (25°C) and exposed for 10 min to a weak far-red or red adaptation beam to adapt them to State I or State II, respectively. The adaption beam was then replaced by an antagonistic beam (i.e., far-red by red or red by far-red) to induce transitions to the alternative state. After different periods of exposure to this second beam, the cells were placed in the dark for 5 s to allow any closed PS II traps to re-open. The chlorophyll fluorescence induction curve was then measured using the saturating actinic beam. The photomultiplier output was captured and digitised by a transient recorder and the areas above the induction curves determined using an Apple 2+ microcomputer. Incident light intensities were measured with a LI-190SB Quantum Sensor (LI-

COR, Lincoln, NE). Redox potentials were monitored using a EIL redox-electrode according to the method of Horton et al. [8].

3. RESULTS AND DISCUSSION

The area above the chlorophyll fluorescence induction curve of algal cells and higher plant chloroplasts is proportional to the number of oxidising equivalents in the electron carrier pool between PS II and PS I [10]. Typical fluorescence induction curves obtained for State I- and State II-adapted *Chlorella* cells are presented in fig.2a. Steihl and Witt [12], working with broken spinach chloroplasts, found that part of the PQ pool was rapidly re-oxidised following exposure to high light intensities. To check that no such oxidation was occurring during the 5-s dark period between pre-adaptation and the measurement of fluorescence induction in the present experiments, the areas above induction curves of samples illuminated with weak red, or far-red light during this period were also measured. No significant differences were observed. The possibility of re-oxidation of PQ by atmospheric O₂ [13] was also investigated by comparing induction curves of algal samples continuously flushed with N₂ with those of aerobic controls. Again the curves were essentially identical. In contrast, addition of sodium dithionite led to marked decreases in the area above the induction curve indicating that the PQ pool could be more fully reduced by the addition of exogenous

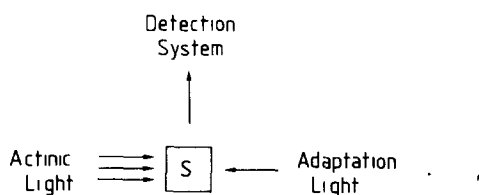


Fig.1. Schematic representation of apparatus used for fluorescence measurements. Samples (S) were pre-illuminated using the adaptation beam (650 nm; $1.56 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ or 706 nm; $9.6 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) isolated from a 500 W tungsten filament lamp using Balzer B-40 interference filters. The actinic light (broad-band blue; $380 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) used to excite fluorescence was isolated from a 250 W quartz-iodide lamp using BG-30 and E-1 Schott filters. The detection system consisted of a Bausch and Lomb monochromator set at 695 nm, an EMI 9658R photomultiplier and a Datalab DL902 transient recorder set at a 5 kHz sampling rate.

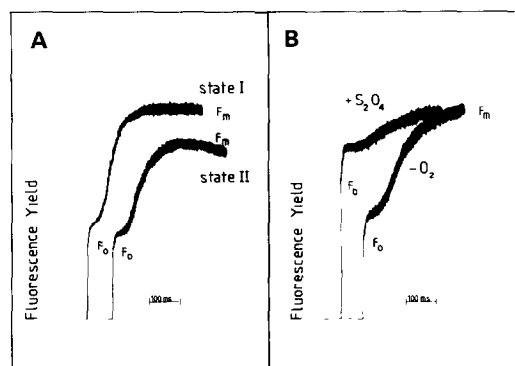


Fig.2. Fluorescence induction curves obtained from: (A) State I- and State II-adapted algae; (B) algae bubbled with N₂ in the presence (+ S₂O₄) and absence (- O₂) of 7.5 mM sodium dithionite.

reductants. Typical induction curves for O_2 -depleted samples and samples containing 7.5 mM sodium dithionite (which leads to a change in the redox potential of the system from about +20 mV to -485 mV) are shown in fig.2b.

The maximal fluorescence yield (F_m) of State I-adapted cells was normally about 20% higher than that of State II-adapted cells reflecting the increased fraction of incident light energy reaching PS II under State I conditions. In order to make direct comparisons of the redox state of the PQ pool in the two states, it is necessary to compensate for this by normalisation of the fluorescence signals to the same variable fluorescence yield F_v [10]. The normalised values of the relative areas above the induction curves of a series of State I- and State II-adapted samples, together with the corresponding values for O_2 -depleted and dithionite-treated algae are listed in table 1. The most significant feature of these values is that the areas obtained for the State I- and State II-adapted cells differ by only about 20% indicating that the difference in the redox state of their PQ pools is relatively small. This presumably reflects the fact that the algae are adapted so as to balance the input of excitation energy into the two photosystems as closely as possible under the prevailing light conditions.

To observe the maximal possible fluctuations in the redox state of the pool, a second series of experiments was performed in which the cells were

Table 1

The effects of State I, State II adaptation and anaerobiosis on the relative areas above the fluorescence induction curves of *Chlorella* cells

Treatment	Relative areas above induction curve ^a
State I-adapted (aerobic)	100 (± 9.9)
State II-adapted (aerobic)	78.6 (± 4.7)
Far-red pre-illuminated (N_2 -bubbled)	98.8 (± 1.5)
Far-red pre-illuminated (7.5 mM dithionite)	34.1 (± 1.0)

^a State I, State II and N_2 -bubbled values normalised to F_v ; dithionite-treated to F_m . Figures in brackets are standard deviations for n samples, where $n = 18, 8, 3$ and 3 for the four treatments

first adapted to a given state and then exposed to an antagonistic light to induce a state change. The redox state of the PQ pool was then determined at different times during the course of the ensuing transition. Plots of the observed changes in relative areas above the fluorescence induction curves and F_m values during these transitions are presented in fig.3.

As would be anticipated, exposure of State I-

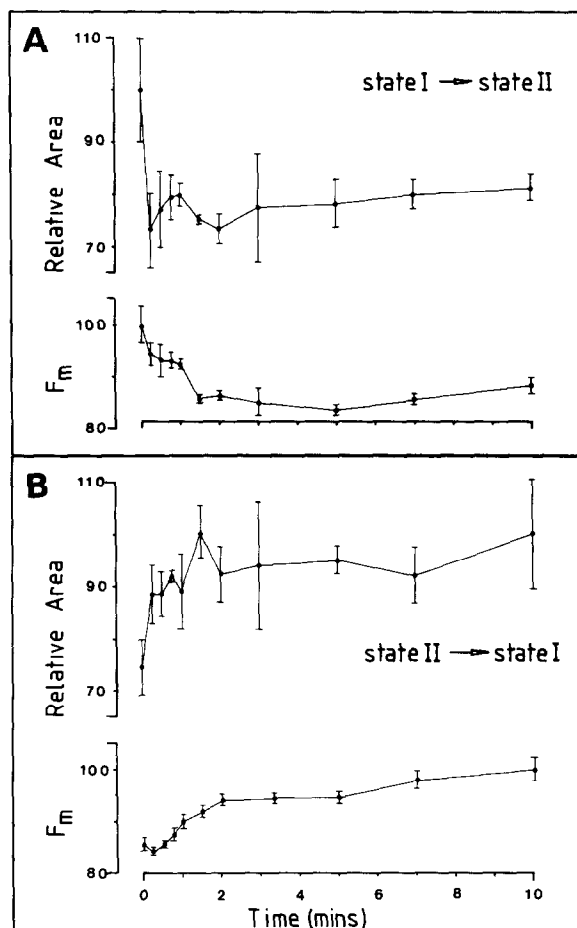


Fig.3. Changes in the relative areas, normalised to F_v , above the fluorescence induction curves and F_m values of *Chlorella* cells during (A) State I → State II and (B) State II → State I transitions, measured as a function of time of illumination in red (650 nm) or far-red (706 nm) light, respectively. Each value represents the mean of three measurements on different samples; the error bar shows their standard deviations. Measurements on different samples were normalised to a constant F_m following adaptation to 706 nm light to minimise between sample variance.

adapted cells to red light resulted in a reduction of the PQ pool and a corresponding decrease in the area over the induction curves. This is essentially complete within 15 s. The extent of this change, about 25%, is, however, again relatively small. The value of F_m for these samples decreased rapidly over a period of about 90 s after which only small changes were apparent. Exposure of State II-adapted cells to far-red light led to an oxidation of PQ and a 20–30% increase in the area above the induction curves that was completed within 60–90 s. The corresponding values of F_m increased much more slowly over a period of 10 min or more, reflecting the much slower rates of State II–State I as opposed to State I–State II transitions in *Chlorella* [14].

It is clear from these measurements that whilst the directions of the observed changes in the redox state of the PQ pool are consistent with the idea that they are associated with the control of excitation energy distribution, the pool remains close to its equilibrium oxidation level at all times. It does not fluctuate to anywhere near the extent seen in *in vitro* redox-poising experiments [8]. The absence of any large fluctuations can readily be accounted for in terms of the postulated role of PQ as a mobile carrier ferrying electrons between PS II, located in the appressed, and PS I, located in the non-appressed, regions of the thylakoid membrane [14,15]. For electron transport to proceed, a balanced diffusion of reduced PQ (PQ_{red}), from PS II to PS I, and oxidised PQ (PQ_{ox}), from PS I to PS II, must occur as illustrated diagrammatically in fig.4. Any major departures from the equilibrium oxidation level of PQ would result in a decrease in PS I or PS II reaction centre turnover and a concomitant decrease in electron transport.

These constraints of *in vivo* electron transport on the redox state of the PQ pool are ignored in redox-poising experiments where LHCP phos-

phorylation is brought about by exogenous ATP. Our results suggest that it is the redox state of other more specialised molecules with redox properties similar to those of the PQ pool, rather than that of the PQ pool as a whole, that is important in the control of the LHCP kinase. Activation of the kinase is known to be prevented by concentrations of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) which block electron flow from PS II to PQ [5]. This suggests that the LHCP kinase is controlled by molecules located on the PS II side of the PQ pool, probably at some point between the single electron acceptors close to the PS II reaction centre and the main part of the PQ pool.

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REFERENCES

- [1] Bonaventura, C. and Myers, J. (1969) *Biochim. Biophys. Acta* 189, 366–383.
- [2] Murata, N. (1969) *Biochim. Biophys. Acta* 189, 171–181.
- [3] Telfer, A. and Barber, J. (1981) *FEBS Lett.* 129, 161–165.
- [4] Bennett, J., Steinbeck, K.E. and Arntzen, C.J. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5253–5257.
- [5] Allen, J.F., Bennett, J., Steinbeck, K.E. and Arntzen, C.J. (1981) *Nature* 291, 25–29.
- [6] Kyle, D.J., Stachelin, L.A. and Arntzen, C.J. (1983) *Arch. Biochem. Biophys.* 222, 527–541.
- [7] Simpson, D.J. (1983) *Biochim. Biophys. Acta* 725, 113–120.
- [8] Horton, P., Allen, J.F., Black, M.J. and Bennett, J. (1981) *FEBS Lett.* 125, 193–196.
- [9] Williams, W.P., Furtado, D. and Nutbeam, A.R. (1980) *Photobiophys. Photobiophys.* 1, 91–102.
- [10] Malkin, S. and Kok, B. (1966) *Biochim. Biophys. Acta* 126, 413–432.
- [11] Malkin, S. (1966) *Biochim. Biophys. Acta* 126, 433–442.
- [12] Steihl, H.H. and Witt, H.T. (1969) *Z. Naturforsch.* 24b, 1588–1598.
- [13] Bennoun, P. (1982) *Proc. Natl. Acad. Sci. USA* 79, 4352–4356.
- [14] Catt, M., Saito, K. and Williams, W.P. (1984) *Biochim. Biophys. Acta*, in press.
- [15] Andersson, B. and Anderson, J.M. (1980) *Biochim. Biophys. Acta* 593, 427–440.
- [16] Anderson, J.M. (1981) *FEBS Lett.* 124, 1–10.

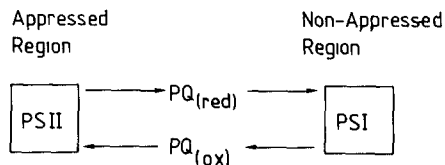


Fig.4. Diagram illustrating the requirement for balanced flow of PQ_{red} and PQ_{ox} between the two photosystems to maintain efficient electron transport. See text for further details.