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State 1–State 2 transitions in the cyanobacterium *Synechococcus* 6301 are controlled by the redox state of electron carriers between Photosystems I and II

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Abstract

The mechanism by which state 1–state 2 transitions in the cyanobacterium *Synechococcus* 6301 are controlled was investigated by examining the effects of a variety of chemical and illumination treatments which modify the redox state of the plastoquinone pool. The extent to which these treatments modify excitation energy distribution was determined by 77 K fluorescence emission spectroscopy. It was found that treatment which lead to the oxidation of the plastoquinone pool induce a shift towards state 1 whereas treatments which lead to the reduction of the plastoquinone pool induce a shift towards state 2. We therefore propose that state transitions in cyanobacteria are triggered by changes in the redox state of plastoquinone or a closely associated electron carrier. Alternative proposals have included control by the extent of cyclic electron transport around PSI and control by localised electrochemical gradients around PSI and PSII. Neither of these proposals is consistent with the results reported here.

Abbreviations: DBMIB – 2,5-dibromo-3methyl-6-isopropyl-*p*-benzoquinone, Chl – chlorophyll, DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DQH₂ – duroquinol (tetramethyl-*p*-hydroquinone), LHC II – light-harvesting chlorophyll *a/b*-binding protein of PS II, Light 1 – light predominantly exciting PSI, Light 2 – light predominantly exciting PS II, M.V. – methyl viologen, PS – photosystem

Introduction

In many photosynthetic organisms the distribution of excitation energy between PS II and PS I may be altered as a rapid response to changes in illumination conditions (Murata 1969, Bonaventura and Myers 1969). These changes are known as state 1–state 2 transitions. State transitions or comparable phenomena have been observed in a wide range of photosynthetic organisms, including green plants (Bonaventura and Myers 1969), red algae

(Murata 1969), cyanobacteria (Fork and Satoh 1983) and cryptomonad algae (Snyder and Biggins 1987). In green plants state transitions result in changes in the distribution of the light-harvesting chlorophyll *a/b*-binding protein LHC II (Staehelein and Arntzen 1983). In state 1, LHC II is associated primarily with PS II. On transition to state 2, a proportion of LHC II becomes functionally decoupled from PS II and migrates into the stromal lamellae of the thylakoid membrane (Staehelein and Arntzen 1983). This may result in increased energy

transfer to PSI. The redistribution of LHCII on transition to state 2 occurs as a result of protein phosphorylation (Allen et al. 1981).

Fluorescence induction transients indicate that state transitions in cyanobacteria involve changes in the efficiency of energy transfer from the phycobilisome to PSII (Mullineaux and Allen 1988). On transition to state 2 a proportion of PSII core complexes become functionally decoupled from the phycobilisomes (Mullineaux and Allen 1988). As in green plants, state transitions in cyanobacteria are accompanied by changes in the phosphorylation state of polypeptides (Allen et al. 1985, Sanders and Allen 1987).

State transitions occur in response to imbalances in the rates of turnover of PSI and PSII (Murata 1969, Bonaventura and Myers 1969). The initiation of a state transition therefore requires a mechanism capable of sensing a change in the turnover rates of the photosystems. Such a mechanism could plausibly be related either to the redox state of an electron carrier or to the transthylakoid electrochemical gradient, since both of these could change rapidly in response to changes in the turnover rates of the photosystems, Murata (1969) and Duysens (1972) suggested that the redox state of an electron carrier located between PSII and PSI could be the controlling factor; this would respond in opposite ways to PSII turnover and PSI turnover.

There is now strong evidence that state transitions in green plants are controlled by the redox level of an electron carrier intermediate between the two photosystems. The reduction of this electron carrier results in the activation of a membrane-bound protein kinase which catalyses the phosphorylation of LHCII. The oxidation of the electron carrier causes the inactivation of the kinase (Allen et al. 1981, Allen and Horton 1981). The electron carrier involved appears to have a redox midpoint potential of about 0 mV, which suggests that it is plastoquinone (Horton et al. 1981). However, recent results suggest that a quinone binding site on the cytochrome *b₆f* complex is involved in the control of state transitions (Gal et al. 1987, Wollman and Lemaire 1988, Bennett et al. 1988).

By contrast, the mechanism by which state transitions in phycobilisome-containing organisms are controlled remains controversial. Murata (1969) and Ried and Reinhardt (1980) proposed that state transitions in red algae were controlled by the

redox state of an intersystem electron carrier as they are now generally accepted to be in green plants. However, Satoh and Fork (1983) proposed that state transitions in a cyanobacterium were controlled by the extent of cyclic electron transport around PSI, the state 1 transition being induced by high rates of cyclic electron transport, Biggins et al. (1984) suggested that state transitions in phycobilisome-containing organisms were induced by localised electrochemical gradients around PSII and PSI. According to their model, turnover of PSI generates a localised change in charge distribution which in turn leads to a small conformational change which produces the functional effects of a state 1 transition. The state 2 transition is similarly induced by localised electrochemical gradients around PSII (Biggins et al. 1984).

In addition to the photosynthetic electron transport chain, the thylakoid membranes of cyanobacteria contain a respiratory electron transport chain that catalyses electron transport from NAD(P)H to oxygen [see Scherer et al. (1988) for a review]. The redox level of the plastoquinone pool involved in photosynthetic electron transport can be perturbed by the activity of the respiratory electron transport chain, indicating that the two electron transport chains intersect and share a common plastoquinone pool (Hirano et al. 1980, Aoki and Katoh 1982).

The state 2 transition in the cyanobacterium *Synechococcus* 6301 can be driven by respiratory electron flow into the plastoquinone pool as well as by PSII turnover (Mullineaux and Allen 1986, Dominy and Williams 1987). This is most easily explained on the assumption that state transitions in cyanobacteria are controlled by the redox level of plastoquinone or an associated electron carrier. However, it is possible that respiratory electron transport could inhibit cyclic electron flow around PSI by reducing components of the cyclic electron transport pathway. The proposal of Satoh and Fork (1983) that the state 1 transition is induced by cyclic electron transport may also therefore be consistent with these results.

Here we present results intended to distinguish between these possibilities. We have investigated the effects on excitation energy distribution in the cyanobacterium *Synechococcus* 6301 of a variety of reagents which perturb the electron transport chain. We have used 77 K fluorescence emission

spectroscopy to assess the extent of adaptation of cells to state 1 or to state 2: this technique has been widely used in both phycobilisome-containing organisms (Murata 1969, Fork and Satoh 1983, Bruce et al. 1985) and green plants (Krause and Behrend 1983). Cells frozen in liquid nitrogen retain patterns of excitation energy distribution characteristic of the light-state to which they were adapted prior to freezing; illumination after freezing does not perturb the light-state of the cells (Murata 1969). This is an advantage of 77 K spectroscopy over room-temperature fluorescence measurements, since at room temperature the measurement will tend to perturb the state of the cells. Additional advantages of 77 K fluorescence spectroscopy are that the fluorescence peaks are more sharply resolved than at room temperature and that at low temperature there is significant fluorescence emission from PSI [see Krause and Weis (1984) for a review]. This allows state transitions to be distinguished from other processes that alter the chlorophyll fluorescence yield (Krause and Behrend 1983, Krause et al. 1983).

Here we show that treatments that cause the plastoquinone pool to become more reduced induce a shift towards state 2 whereas treatments that cause the plastoquinone pool to become more oxidised induce a shift towards state 1. The predicted effects of these treatments according to a number of other models for the control of state transitions in phycobilisome-containing organisms are also considered. Several of these alternative models successfully predict the effect of a number of these treatments, but none of them are consistent with all the results reported here. We therefore conclude that state transitions in cyanobacteria are controlled by the redox level of plastoquinone or by that of a closely associated electron carrier.

Materials and methods

Cells of *Synechococcus* 6301 were grown in liquid culture in Medium C of Kratz and Myers (1955). The cells were grown at 35°C under illumination with warm-white fluorescent lamps at an intensity of about 10 W m⁻². The cells were bubbled with a mixture of 5% CO₂ and 95% N₂. Cells were grown to the late-logarithmic phase of growth. They were then subcultured to a concentration of about 1 µg

Chl *a*, ml⁻¹ in fresh Medium C and grown for a further 4–5 h at a slightly increased light intensity of about 15 W m⁻². This procedure was found to give cells which consistently performed large and rapid state transitions.

The cells were harvested by centrifugation and resuspended in Medium C to a concentration of 5 µg Chl *a*, ml⁻¹. Where appropriate, reagents were then added and 60 µl samples of the cell suspension were loaded into glass capillary tubes. The cells were then exposed to light of the specified intensity and spectral quality for 5 min before being rapidly frozen by plunging into liquid nitrogen. In most cases the reagent was added and the cell sample loaded into the tube immediately before commencing the light incubation. In the case of methyl viologen, however, the cells were incubated in the dark for 15 min in the presence of the reagent prior to the light adaptation. DCMU was added as a 10 mM solution in ethanol and DBMIB was added as a 0.1 mM solution in ethanol. Methyl viologen and KCN were added as 100 mM solutions in water. Duroquinol was freshly prepared: a 50 mM solution of duroquinone in ethanol was reduced with sodium borohydride and a small volume of concentrated hydrochloric acid was subsequently added to remove excess borohydride (Allen and Horton 1981). All incubations were performed at room temperature (20°C). The frozen samples were stored in the dark in liquid nitrogen.

Prior to freezing the cells were adapted to either blue, yellow, or far-red light. The light source used was a stabilised 250 W projector lamp. Blue light was defined by a combination of Corning 5–60 and Corning 4–96 glass filters, giving maximum transmission at 425 nm. Yellow light was defined by a combination of a Corning 4–96 glass filter and an Ealing 560 nm long-wavelength band-pass filter: this filter combination gave maximum transmittance at 570 nm. Far-red light was defined by an Ealing 709 nm interference filter. Light intensities were controlled with Schott neutral density filters.

Fluorescence emission spectra were recorded at 77 K with a Perkin–Elmer LS–5 luminescence spectrometer. The excitation monochromator was set to either 600 nm or 435 nm, as indicated, with a 2.5 nm slitwidth. Emission was scanned from 630 nm to 750 nm. The slitwidth for the emission monochromator was 5 nm. Samples were exposed to the excitation beam for 2–3 min before recording

the spectrum. All spectra were normalised to the phycocyanin fluorescence emission peak (excitation at 600 nm, emission at 654 nm). Spectra recorded with excitation at 435 nm were corrected for varying amounts of light scattering by aligning the 630–670 nm region of the spectrum. All comparisons of spectra were made between samples from the same batch of cells.

Results

Effect of state transitions on 77 K fluorescence emission spectra

Figure 1 shows 77 K fluorescence emission spectra for cells of *Synechococcus* 6301 adapted to far-red light or dark. Far-red light preferentially excites PSI and therefore induces a state 1 transition whereas cyanobacteria tend to revert to state 2 in the dark (Fork and Satoh 1983). The spectra show

four main fluorescence peaks. The peak at 654 nm comes from phycocyanin, the peak at 685 nm comes both from the phycobilisome terminal emitter and from the small chlorophyll *a* antenna of PS II, the peak at 697 nm comes from PS II and the peak at 720 nm comes from PSI (Bruce et al. 1985). With excitation at 600 nm (Fig. 1A), which is absorbed primarily by the phycobilisome, the 654 nm peak predominates but there is also substantial fluorescence from the PS II fluorescence peaks at 685 nm and 697 nm. Little fluorescence is seen from PSI which is only weakly excited by yellow light (Manodori and Melis 1986). The 720 nm fluorescence peak is accordingly reduced to a shoulder on the PS II fluorescence emission peak (Fig. 1A). With excitation at 435 nm (Fig. 1B) which is absorbed predominantly by chlorophyll *a*, the phycocyanin fluorescence emission peak at 654 nm is very small. There is, however, substantial fluorescence from PS II at 685 nm and 697 nm due to absorption of 435 nm light by the small PS II chlorophyll

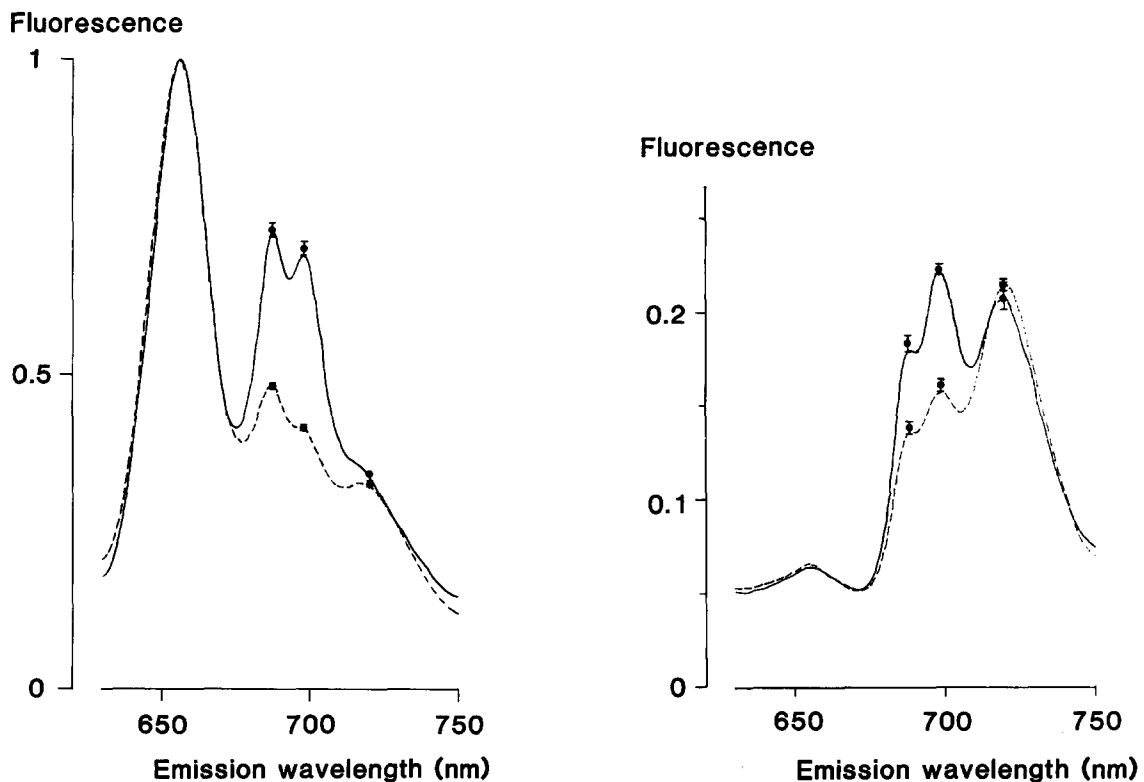


Fig. 1. Low temperature fluorescence emission spectra for cells of *Synechococcus* 6301 in state 1 and state 2. A. Excitation at 600 nm. B. Excitation at 435 nm. Solid line: Cells in state 1 (adapted to far-red light). Broken line: Cells in state 2 (dark-adapted). Spectra recorded at 77 K as described in Materials and Methods. Dots and error bars indicate average peak heights and standard errors from three samples.

antenna. With excitation at 435 nm (Fig. 2B, 3) a major proportion of the observed fluorescence comes from the PSI peak at 720 nm.

The spectra for red-light adapted cells and dark-adapted cells show differences characteristic of the changes in excitation energy distribution which result from state transitions (Murata 1969, Ley and Butler 1980, Fork and Satoh 1983, Bruce and Biggins 1985). Cells adapted to far-red light show greatly increased PS II fluorescence as compared to dark-adapted cells. This change is seen both with phycobilisome-absorbed excitation light (Fig. 1A) and with chlorophyll-absorbed excitation light (Fig. 1B). In both cases the fluorescence increase is specific to the PS II fluorescence peaks.

Dependence of the state 1 transition on light intensity and quality

Figure 2 compares the effects of exposure to different intensities of blue and yellow light on excitation energy distribution as determined by 77 K fluorescence emission spectroscopy. The yellow light is absorbed primarily by phycocyanin and therefore preferentially excites PS II, the blue light is absorbed primarily by chlorophyll and therefore preferentially excites PSI. We have used a blue

rather than a far-red light as an example of a light which preferentially excites PSI because light at wavelengths longer than 700 nm is only weakly absorbed by the cell (Manodori and Melis 1986). The dependence of state transitions on the intensity of far-red light therefore cannot usefully be compared to their dependence on the intensity of yellow light, which is strongly absorbed by the cell.

The 77 K fluorescence emission spectra of dark-adapted cells show the low PS II fluorescence emission characteristic of state 2 (Fig. 1). Figure 2 shows that no intensity of blue or yellow light induces a measurable further transition to state 2. At intensities greater than about $1 \mu\text{E m}^{-2} \text{s}^{-1}$ both blue and yellow light induce a shift towards state 1 in dark-adapted cells. However, blue light is clearly considerably more effective than yellow light: a 50% transition to state 1 can be driven by blue light at an intensity of about $4 \mu\text{E m}^{-2} \text{s}^{-1}$ whereas yellow light at an intensity of about $40 \mu\text{E m}^{-2} \text{s}^{-1}$ is required (Fig. 2). This confirms that the state 1 transition is induced specifically by PSI turnover (Murata 1969, Ried and Reinhardt 1980).

The intensity of light required to induce the state 1 transition varied between different batches of cells (compare Figs. 2 and 3). A possible explanation would be that the rate of respiratory electron transport differed between different batches of cells; res-

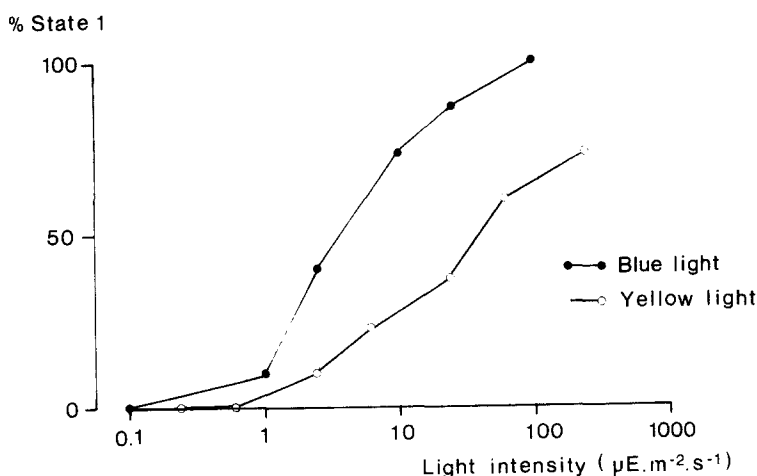


Fig. 2. Dependence of the state 1 transition in *Synechococcus* 6301 on light intensity and quality. Cells of *Synechococcus* 6301 were adapted for 5 min to light at the intensity indicated before being frozen. The combination of filters used to define the yellow light had maximum transmittance at 570 nm. The combination of filters used to define the blue light had maximum transmittance at 425 nm. The extent of adaptation to state 1 was determined from 77 K fluorescence emission spectra recorded with excitation at 600 nm, the ratio F_{697}/F_{654} being used as an indicator of light-state. The ratio F_{697}/F_{654} for cells adapted to blue light at $100 \mu\text{E m}^{-2} \text{s}^{-1}$ was 0.68: this was taken to correspond to 100% state 1. The ratio F_{697}/F_{654} for dark-adapted cells was 0.38: this was taken to correspond to 0% state 1.

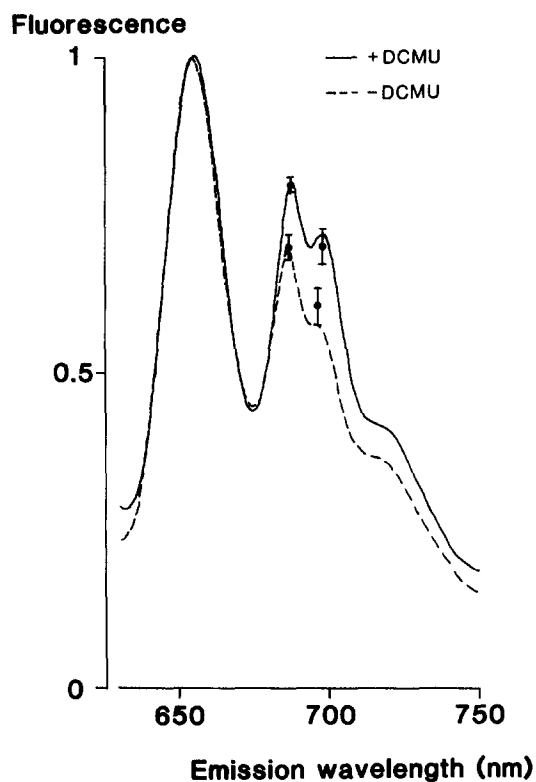
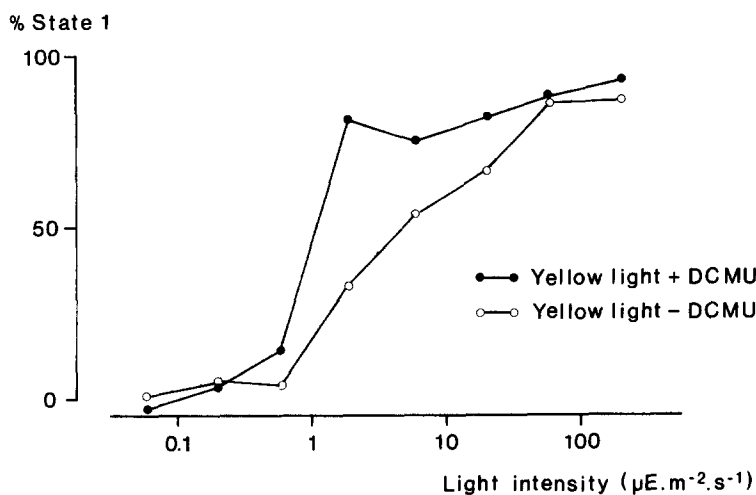


Fig. 3. Effect of DCMU on light state in *Synechococcus* 6301. A. Dependence of the state 1 transition on light intensity with and without $10\ \mu\text{M}$ DCMU, yellow light was defined by filter combination with maximum transmittance at $570\ \text{nm}$. The extent of adaptation to state 1 was determined from the ratio F_{697}/F_{654} in $77\ \text{K}$ fluorescence emission spectra recorded with excitation at $600\ \text{nm}$, by comparison with the spectra for cells adapted to $709\ \text{nm}$ light at $180\ \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (100% state 1) and dark-adapted (0% state 1). B. $77\ \text{K}$ fluorescence emission spectra for cells adapted to $570\ \text{nm}$ light at $2.4\ \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with and without $10\ \mu\text{M}$ DCMU. Excitation was at $600\ \text{nm}$. Dots and error bars indicate average peak heights and standard errors from four replicates.

piratory electron flow into the plastoquinone pool induces the state 2 transition and inhibits the state 1 transition (Mullineaux and Allen 1986, Dominy and Williams 1987). To surmount this difficulty, we have made comparisons only between samples of cells from the same batch.

Effect of DCMU on light-state

Figure 3 shows the effect of DCMU on the intensity-dependence of the state 1 transition induced by yellow light. DCMU blocks the transfer of electrons from PS II to plastoquinone, thereby preventing PSII turnover. Figure 3A indicates that the ability of yellow light to drive a state 1 transition is enhanced by the presence of DCMU: the state 1 transition can be induced by lower intensities of light when DCMU is present, although there is little change in the extent of the state 1 transition induced by high intensities of light.

To confirm that the effect of DCMU is significant we repeated the experiment on a separate sample of cells using only one light intensity ($2.4 \mu\text{E m}^{-2}\text{s}^{-1}$) but freezing 4 replicate samples of cells adapted to yellow light with and without DCMU. The spectra for these samples are shown in Fig. 3B. The presence of DCMU causes a significant shift towards state 1. There could in principle be two reasons for this effect:

- The state 1 transition could be inhibited by PS II turnover. DCMU prevents PS II turnover and therefore enhances the state 1 transition.
- DCMU causes PS II centres to become closed in the light. Under these conditions the yield of processes which compete with PS II photochemistry is enhanced. One of these processes could be spillover of excitation energy to PSI. The presence of DCMU could therefore increase the effective absorption cross-section of PSI, thus increasing PSI turnover and hence the state 1 transition even if PS II turnover has no direct effect on the state 1 transition. It should be noted however that there is evidence that little or no energy absorbed by the phycobilisome is subsequently transferred to PSI, indicating that the efficiency of spillover of excitation energy from phycobilisome-coupled PS II centres is low (Manodori et al. 1984).

Effect of DBMIB on light-state

Figure 4 shows the effect of exposure to a high intensity of far-red light in the presence and in the absence of DBMIB. DBMIB inhibits electron transfer from plastoquinone to the Cytochrome complex (Trebst and Harth 1970). The presence of DBMIB at $2 \mu\text{M}$ was sufficient completely to suppress light-dependent oxygen evolution in cells of *Synechococcus* 6301 (results not shown). DBMIB at concentrations higher than $2 \mu\text{M}$ significantly inhibits electron transfer from PS II to plastoquinone in isolated chloroplasts (Trebst and Harth 1970).

DBMIB at $2 \mu\text{M}$ completely prevents the red-light induced state 1 transition in *Synechococcus* 6301 (Fig. 4). Unlike DCMU (Fig. 3A) the effect of DBMIB on the state 1 transition occurs over a full

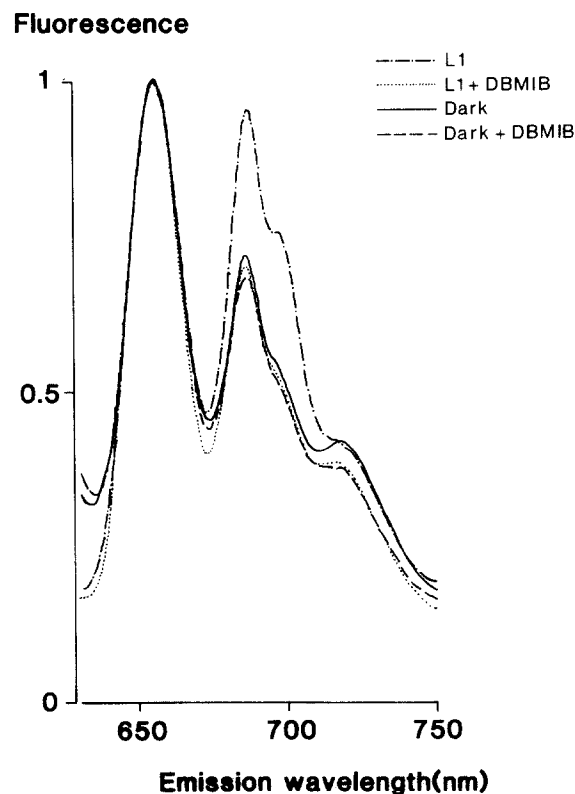


Fig. 4. Effect of DBMIB in light-state in *Synechococcus* 6301. Cells were adapted for 5 min to Light 1 (defined by a 709 nm interference filter at an intensity of $180 \mu\text{E m}^{-2}\text{s}^{-1}$) or dark, with and without DBMIB at $2 \mu\text{M}$. Fluorescence emission spectra were recorded at 77 K with excitation at 600 nm.

range of light intensities (not shown). The result indicates that PSI-dependent electron flow from plastoquinone to the cytochrome *b₆f* complex is an essential stage in the induction of the state 1 transition by light.

Effect of duroquinol on light-state

Figure 5 shows the effect of exposure to a high intensity of far-red light in the presence and in the absence of duroquinol (tetramethyl-*p*-hydroquinone). Duroquinol is a lipid-soluble reducing agent whose presence causes the plastoquinone pool to become more reduced. However, the efficiency of electron transfer from duroquinol to plastoquinone appears to be rather low, and it seems likely that duroquinol acts principally as a direct electron donor to a quinone binding site on the cytochrome *b₆f* complex [Nanba and Katoh, 1986]. The ability of duroquinol to donate electrons to the cytochrome *b₆f* complex in intact cyanobacterial cells has been well-established (Nanba and Katch 1986).

In the presence of duroquinol, exposure to far-red light does not induce a significant increase in PS II fluorescence (Fig. 5). This indicates that duroquinol is a strong inhibitor of the state 1 transition. An alternative explanation for the effect of duroquinol would be that PS I turnover during the light-incubation significantly increases the proportion of duroquinone, the oxidised form of duroquinol, in the preparation. Since duroquinone is a direct quencher of fluorescence, this could compensate for the fluorescence increase caused by a state 1 transition. However, duroquinol completely prevented a light-induced PS II fluorescence increase over a full range of light intensities (not shown). It seems highly unlikely that PS I turnover would generate just sufficient duroquinone to compensate for the effects of a state 1 transition at every intensity of light used. Duroquinol also induces a further decrease in PS II fluorescence in dark-adapted cells (Fig. 5). This effect may, however, be due to the presence of duroquinone in the preparation and may not be related to a state 2 transition.

Effect of potassium cyanide on light-state

Figure 6 shows the effect of KCN on excitation energy distribution in cells adapted to dark and to

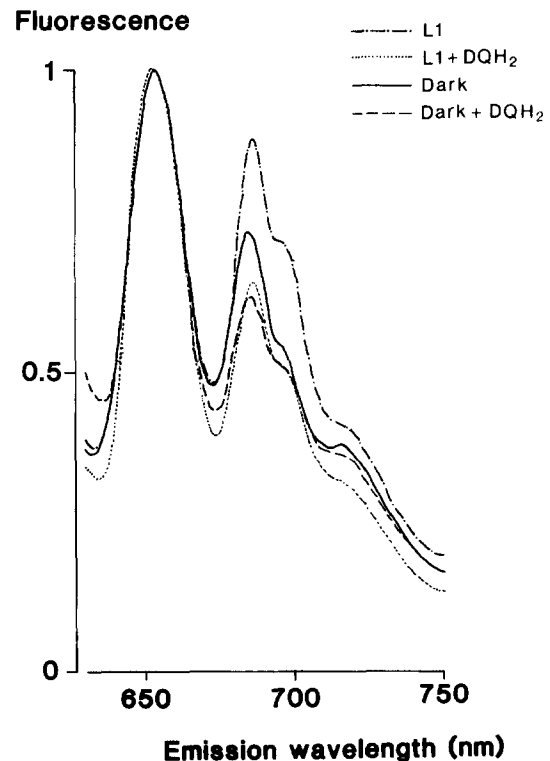


Fig. 5. Effect of duroquinol on light-state in *Synechococcus* 6301. Cells were adapted for 5 min to Light 1 defined by a 709 nm interference filter at an intensity of $180 \mu\text{Em}^{-2}\text{s}^{-1}$ or dark, with and without duroquinol at 0.5 mM. Fluorescence emission spectra were recorded at 77 K with excitation at 600 nm.

yellow light. Under both these conditions KCN induces a small decrease in PS II fluorescence. Although the effect of KCN was small, it was consistently observed and was larger than the estimated standard error of the measurement (Fig. 6). This indicates that KCN inhibits the light-induced state 1 transition and induces a slight further state 2 transition in dark-adapted cells. KCN inhibits photosynthetic electron transport at two points. KCN inhibits cytochrome oxidase, thus preventing respiratory electron flow from metabolites to oxygen in the dark (Hirano et al. 1980). Further, KCN prevents turnover of the Calvin cycle by inhibiting ribulose biphosphate carboxylase. KCN at 1 mM completely inhibited both respiratory oxygen uptake in the dark and oxygen evolution under illumination with yellow light (not shown). The results in Fig. 6 therefore indicate that the inhibition of electron transport away from the plastoquinone pool and the cytochrome *b₆f* complex promotes a shift towards state 2.

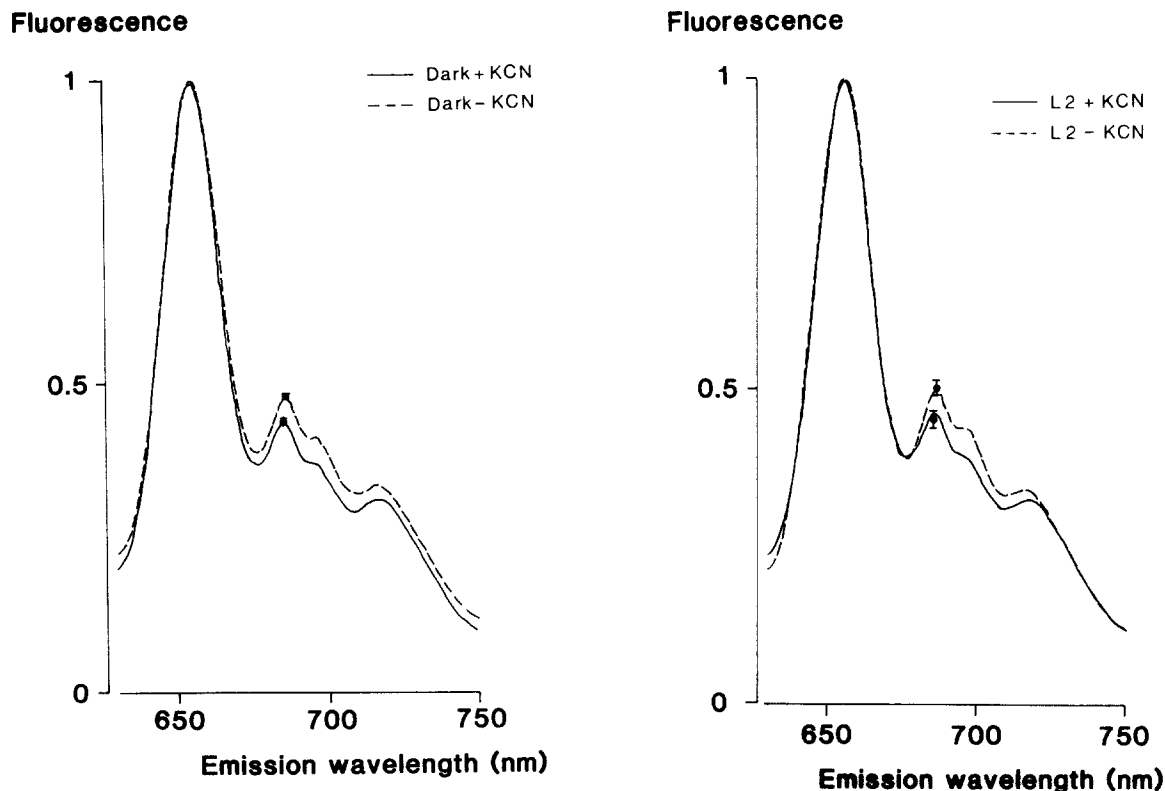


Fig. 6. Effect of potassium cyanide on light-state in *Synechococcus* 6301. Cells were adapted for 5 min to dark (A) or Light 2 (B) with and without KCN at 1 mM. Light 2 was defined by a filter combination with maximum transmittance at 570 nm, and was at an intensity of $2.4 \mu\text{E m}^{-2} \text{s}^{-1}$. Fluorescence emission spectra were recorded at 77 K with excitation at 600 nm. Dots and error bars indicate average 685 nm peak heights and standard errors from three replicates.

Effect of methyl viologen on light-state

Figure 7 shows the effect of methyl viologen on excitation energy distribution in cells adapted to dark and to yellow light. Methyl viologen is a PS I electron acceptor which therefore inhibits cyclic electron transport around PS I by withdrawing electrons from components involved in the cyclic electron transport pathway. It is important to note that methyl viologen will not, under any circumstances, increase the rate of cyclic electron flow around PS I. Cyclic electron transport may be inhibited by over-reduction of electron carriers in the cytochrome b_6/f complex. Under these conditions, methyl viologen may cause the electron carriers to become more oxidised by providing an additional electron acceptor for PS I, but it will not enhance cyclic electron transport because the oxidation of these electron carriers depends on the removal of the electrons that are required for cyclic electron flow.

Methyl viologen can act as a PS I electron acceptor in intact cyanobacterial cells (Chua 1971, Nanba and Katoh 1986). Figure 7 shows that methyl viologen has no significant effect on excitation energy distribution in dark-adapted cells. In cells adapted to far-red light, a strong state 1 transition occurred both in the presence and in the absence of methyl viologen (not shown). In cells adapted to yellow light, however, methyl viologen induces a significant shift towards state 1 (Fig. 7B). The effect of methyl viologen was consistently observed and larger than the estimated error in the measurement (Fig. 7B). This result appears to conflict with that of Satoh and Fork (1983) who found that methyl viologen apparently inhibits the state 1 transition in cells of the cyanobacterium *Synechococcus lividus*. However, Satoh and Fork (1983) assessed the extent of the state 1 transition by observing the size of the slow phase of the fluorescence rise following the addition of a blue 'light 1': in their interpretation of their

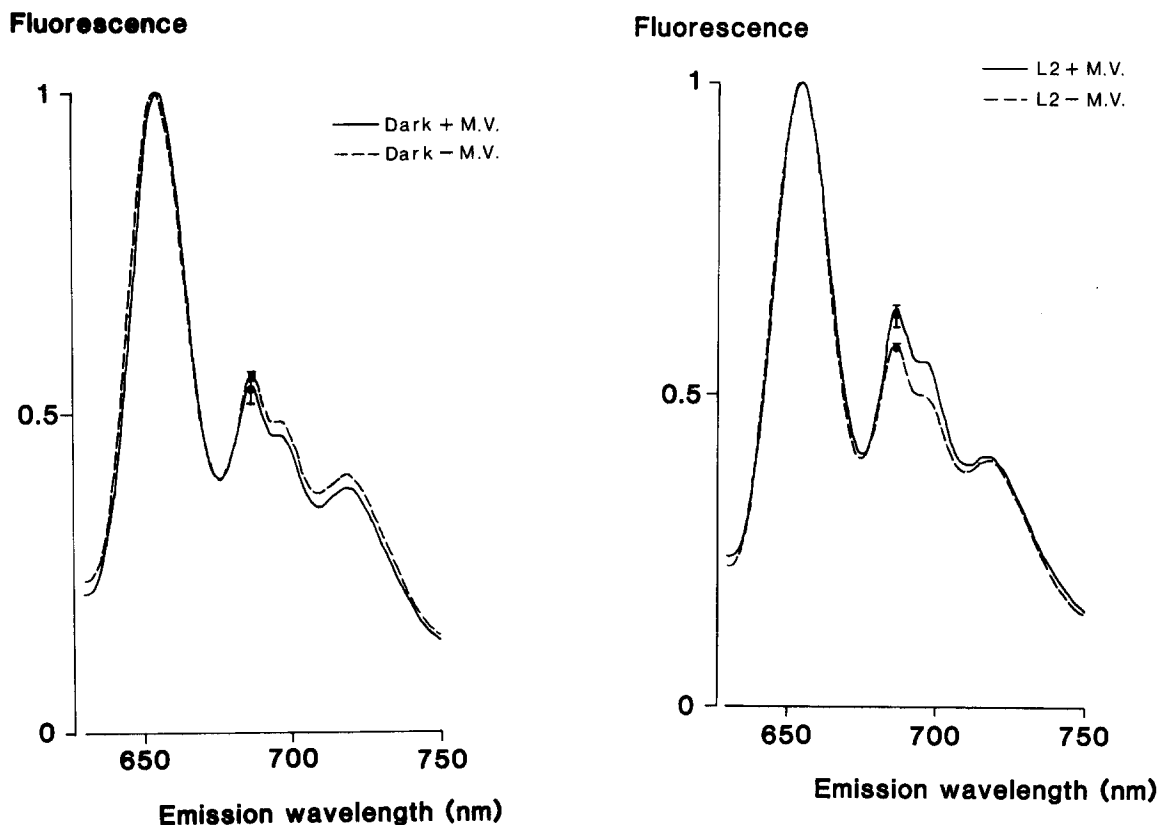


Fig. 7. Effect of methyl viologen on light-state in *Synechococcus* 6301. Cells were adapted for 5 min to dark (A) or Light 2 (B) with and without methyl viologen at 2 mM. Light 2 was defined by a filter combination with maximum transmittance at 570 nm, and was at an intensity of $2.4 \mu\text{E m}^{-2} \text{s}^{-1}$. Fluorescence emission spectra were recorded at 77 K with excitation at 600 nm. Dots and error bars indicate average 685 nm peak heights and standard errors from three replicates.

sults these authors do not allow for the possibility that methyl viologen induces a shift towards state 1 prior to the addition of light 1.

Discussion

The electron transport pathways of cyanobacteria form a complex interacting system where the addition of any reagent is likely to have more than one effect. For this reason the effect of any one reagent does not discriminate between all the possible models for the control of state transitions in cyanobacteria. However, the majority of models for the control of state transitions can be eliminated because they fail correctly to predict the effects of all the reagents used. This is illustrated in Table 1, which compares the observed effects of the reagents

with the effects predicted by a variety of possible models.

The first model considered (A in Table 1) is that of Satoh and Fork (1983). These authors proposed that state transitions in cyanobacteria are controlled by the extent of a transmembrane potential generated by cyclic electron transport around PSI, the state 1 transition being induced by high levels of cyclic electron transport. This model fails to predict the effect of KCN in the dark (Fig. 6); since cyclic electron flow does not occur in the dark KCN should have no effect under these conditions. Furthermore, this model is not consistent with the effects of KCN and methyl viologen in the light. KCN would be expected to stimulate cyclic electron transport around PSI by inhibiting the alternative electron transport routes. Cyclic electron transport might eventually be inhibited by a large transmembrane potential built

Table 1. "Truth table" for the control of state transitions in cyanobacteria. Predicted effects of illumination and electron transport inhibitors on state transitions in cyanobacteria according to the following models. A. The state 1 transition is induced by cyclic electron transport around PSI. B. State transitions are induced by localised electrochemical gradients around PSI and PSII: PSI turnover induces state 1 and PSII turnover induces state 2, but the cells default to state 2 in the dark. C. State transitions are controlled by the redox state of plastoquinone or an associated electron carrier: state 1 is induced when plastoquinone is oxidised and state 2 is induced when plastoquinone is reduced. (+) indicates that the addition of the reagent or illumination specified in the first column would induce a shift towards state 1 in cells adapted to the illumination condition specified in the second column. (–) indicates a shift towards state 2 and (0) indicates no effect. (?) indicates that the model is not specific enough for the effect to be predicted.

Added reagent	Prior condition	Control model			Experimental result
		A	B	C	
Light 1	Dark	+	+	+	+ (Fig. 1)
Light 2	Dark	+	0	–	0 (Fig. 2)
DCMU	Light 2	+	+	+	+ (Fig. 3)
DBMIB	Light 1	–	?	–	– (Fig. 4)
DQH ₂	Dark	0	0	–	– (Fig. 5)
DQH ₂	Light 1	–	+	–	– (Fig. 5)
KCN	Dark	0	0	–	– (Fig. 6)
KCN	Light 2	+	?	–	– (Fig. 6)
M.V.	Light 2	–	+	+	+ (Fig. 7)

up in the absence of Calvin cycle turnover. However, since it is this transmembrane potential which has been postulated to trigger the state 1 transition (Satoh and Fork 1983), KCN must enhance the state 1 transition according to model A. However, KCN inhibits the light-induced state 1 transition (Fig. 6B). Methyl viologen will inhibit cyclic electron transport by withdrawing electrons from components involved in the pathway. According to model (A) methyl viologen would therefore be expected to inhibit the state 1 transition: in fact methyl viologen enhances the light-induced state 1 transition (Fig. 7B). In connection with the model of Satoh and Fork (1983) it should also be noted that the importance of cyclic electron transport around PSI in cyanobacteria has been questioned: there is evidence that indicates that cyclic electron transport does not occur to any significant extent under physiological conditions (Myers 1986, Myers 1987).

Model (B) in Table 1 is that of Biggins et al. (Biggins et al. 1984). These authors proposed that state transitions are induced by localised electrochemical gradients around PSI and PSII, even though the cells apparently revert to state 2 in the dark (Biggins et al. 1984). It is difficult to predict the effects of inhibitors such as DBMIB and KCN according to this model, since both these inhibitors will decrease both PSII and PSI turnover. However, this model is not consistent with the effect of duroquinol (Fig. 5). This reagent is an efficient electron donor to the cyto-

chrome *b₆f* complex (Nanba and Katoh 1986). It would therefore enhance PSI turnover by providing an additional source of electrons to the donor side of PSI and inhibit PSII turnover by competing with plastoquinone for oxidising equivalents at the cytochrome *b₆f* complex. According to the model of Biggins et al. (1984) duroquinol should therefore enhance the light-induced state 1 transition: in fact duroquinol strongly inhibits the light-induced state 1 transition (Fig. 5).

Model (C) in Table 1 postulates that state transitions are controlled by the redox state of plastoquinone or an associated electron carrier, the state 1 transition being induced when the electron carrier is oxidised and the state 2 transition being induced when the electron carrier is reduced (Murata 1969, Ried and Reinhardt 1980) (see Fig. 8). Table 1 shows that this model correctly predicts the effects of all the chemical reagents tested. DCMU and methyl viologen both cause the plastoquinone pool to become more oxidised in the light and would therefore be expected to enhance the state 1 transition according to model (C). Figure 3 and 7B show that these reagents do indeed enhance the state 1 transition in the light.

DBMIB, duroquinol and KCN will all cause the plastoquinone pool to become more reduced in the light. Table 1 shows that all these reagents inhibit the light-induced state 1 transition as predicted by model (C). KCN also causes a small but significant

shift towards state 2 in dark-adapted cells (Fig. 6A). This is consistent with model (C) since the redox state of plastoquinone in the dark will be determined by the relative rates of respiratory electron flow into the plastoquinone pool and oxidation of the plastoquinone pool by electron transfer through the cytochrome b_6f complex, cytochrome c_{553} and cytochrome oxidase. KCN inhibits cytochrome oxidase and will therefore cause the plastoquinone pool to become more reduced in the dark.

Model (C) in Table 1 fails to predict the observed effect in one instance. Illumination with the yellow 'light 2' should cause the plastoquinone pool to become more reduced since yellow light is absorbed primarily by the phycobilisome and should therefore preferentially drive PS II turnover. Illumination with light 2 should therefore induce a shift towards state 2. In fact, illumination with light 2 at low intensity has no effect, and light 2 at high intensity induces a state 1 transition (Fig. 2). However this apparent anomaly may be explained by two considerations:

- (a) Respiratory electron transport keeps the plastoquinone pool largely reduced in the dark (Aoki and Katoh 1982). The addition of light 2 at low intensity may not therefore cause significant further reduction of the plastoquinone pool.
- (b) At high light intensities yellow light acts as light 1 rather than as light 2 in cyanobacteria. Illumination with yellow light at high intensity causes the oxidation of both the plastoquinone pool (Aoki and Katoh 1982) and the cytochrome b_6f complex (Myers 1986). This is probably a consequence of the low PS II/PS I ratio in cyanobacteria (Aoki and Katoh 1982).

For these reasons, the addition of yellow light may not cause the plastoquinone pool to become more reduced, so the effects of yellow light are consistent with the control of state transitions by the redox level of plastoquinone. The inhibition of the state 1 transition by PS II turnover which this model predicts may be inferred from the effect of DCMU. DCMU inhibits PS II turnover and enhances the state 1 transition (Fig. 3).

The ability of PS II-absorbed light to drive a state 2 transition has been well-documented in red algae (Murata 1969, Ried and Reinhardt 1980). It is likely that the respiratory electron transport pathway is less active in red algal chloroplasts than in cyanobacteria, so low intensities of light 2 may

cause significant net reduction of the plastoquinone pool in these organisms.

The state 1 transition in intact cyanobacterial cells appears to be inhibited by uncouplers of photophosphorylation (Satoh and Fork 1983). It has been argued that this must indicate that state transitions are controlled by electrochemical gradients rather than by the redox state of an electron carrier such as plastoquinone (Satoh and Fork 1983, Biggins et al. 1984). However, uncouplers are likely to have complex effects on electron transport in whole cells. Firstly, they will abolish the constraints on electron transport imposed by transmembrane potential differences (photosynthetic and respiratory control). They will also deplete the ATP level in the cell, eventually preventing turnover of the Calvin cycle and therefore depleting the pool of PSI electron acceptors. The effect of uncouplers does not therefore rule out control by the redox state of an electron carrier. Uncouplers are known to stimulate LHC II phosphorylation and therefore the state 2 transition in green plants, where redox control of state transitions is well established (Ferryhough et al. 1984).

Fork and Satoh (1983) have argued that state transitions are not influenced by the redox state of the plastoquinone pool since exposure to PS I-absorbed light induces a state 1 transition even in cells starved by prolonged incubation in the dark. In the starved cells, the plastoquinone pool is more oxidised (Fork and Satoh 1983). However, the data presented by Fork and Satoh (1983) do not exclude the possibility that the plastoquinone pool was partially reduced even in starved, dark-adapted cells. The addition of PS I absorbed light could then still cause the plastoquinone pool to become more oxidised. The results of Fork and Satoh (1983) are not therefore inconsistent with control of state transitions by the redox state of the plastoquinone pool, or with the induction of the state 2 transition by respiratory electron flow (Mullineaux and Allen 1986, Dominy and Williams 1987).

Many studies of the control of state transitions in green plants have employed isolated thylakoid membranes. In this system the dark-state is state 1 (due to oxidation of plastoquinone by equilibration with atmospheric potential) and it is the state 2 transition which is actively driven by light. This is particularly the case when no PS I electron acceptor

is provided: the addition of light will then inevitably cause the reduction of the plastoquinone pool due to PS II turnover. In intact cyanobacteria, by contrast, the dark state is normally state 2 due to respiratory electron flow (Mullineaux and Allen 1986) and it is the state 1 transition which is actively driven by light (Fig. 2). When allowances are made for this difference, the effects of electron donors and acceptors and electron transport inhibitors appear to be strikingly similar in cyanobacteria and green plants. In pea chloroplasts, duroquinol promotes LHC-II phosphorylation and a state 2 transition in the dark (Allen and Horton 1981); in cyanobacteria, duroquinol inhibits the light-dependent state 1 transition (Fig. 5). In pea chloroplasts light-dependent LHC-II phosphorylation and therefore the state 2 transition are inhibited by DCMU but not by DBMIB at low concentrations (Allen et al. 1981); in cyanobacteria the state 1 transition is enhanced by DCMU (Fig. 3) but inhibited by DBMIB (Fig. 4). In pea chloroplasts, LHCII phosphorylation is inhibited by methyl viologen (Allen et al. 1981); in cyanobacteria the light-dependent state 1 transition is enhanced by methyl viologen (Fig. 7).

This suggests that the mechanism by which state transitions are triggered in phycobilisome-containing organisms is similar to that in green plants. In both cases state transitions appear to be controlled by the redox level of an intersystem electron carrier (Fig. 8). This electron carrier may be plastoquinone (Horton et al. 1981) or a component of the cytochrome b_6f complex (Gal et al. 1987, Wollman and Lemaire 1988, Bennett et al. 1988). It should be noted that the effect of DBMIB (Fig. 4) does not rule out the involvement of a component of the cytochrome b_6f complex in the control of state transitions, since some components of the cytochrome b_6f complex may be reduced in the presence of DBMIB as a result of the interaction of reduced plastoquinone with the Q_c binding site (Wollman and Lemaire 1988).

The model illustrated in Fig. 8 has interesting implications for the physiological function of state transitions in cyanobacteria. In cyanobacteria PS II is not the only possible source of electrons for linear photosynthetic electron transport. In heterotrophic cyanobacteria electrons may also be obtained from imported substrates including organic compounds such as sugars (Stanier and Cohen-Bazire 1977)

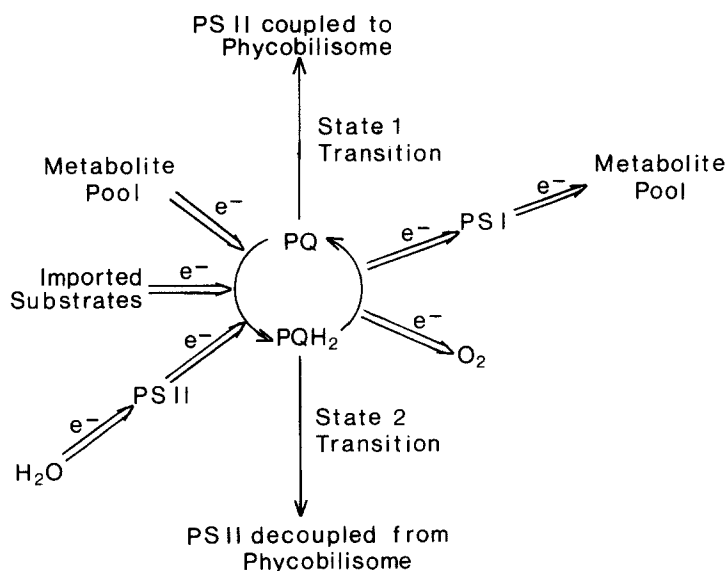


Fig. 8. Model for the control of state transitions in cyanobacteria. The state 1 transition is induced by the oxidation of the plastoquinone pool. This can occur due to PS I turnover or due to the activity of the respiratory cytochrome oxidase. The state 2 transition is induced by the reduction of the plastoquinone pool. The plastoquinone pool can be reduced by PS II turnover or by reducing equivalents obtained from stored or imported metabolites. Reduced plastoquinone may be directly involved in the activation of the mechanism responsible for the state 2 transition. Alternatively, a component of the cytochrome b_6f complex may be involved.

and inorganic compounds such as sulphides (Belkin and Padan 1983). Electrons obtained from these sources are generally transported via components of the photosynthetic electron transport chain (Aoki and Katoh 1982) and may be utilised for PS I-dependent linear electron transport (Belkin and Padan 1983). Reducing equivalents for photosynthetic electron transport may also be obtained from stored metabolites (Scherer and Böger, 1982): this means that there can be an alternative to PS II as an electron source even in obligately photoautotrophic species.

Where there is an alternative to PS II as a source of electrons for PS I-dependent electron transport it seems likely that it will be to the advantage of the cell to utilise the alternative source of electrons. This is because PS II is susceptible to damage: there is a light-induced degradation of the D1 reaction centre polypeptide (otherwise known as the 32 kDa Q_b -binding protein) which must continually be renewed in order to sustain PS II-dependent electron transport (Kyle et al. 1984, Ohad et al. 1984). This damage to the D1 polypeptide occurs specifically when the plastoquinone pool is reduced (Kyle et al. 1984). PS II is therefore likely under such conditions to be rather expensive in metabolic terms as a donor of electrons. State transitions could provide a mechanism for preventing unnecessary PS II turnover: if an alternative source of electrons is available, the plastoquinone pool will become reduced and the state 2 transition will be induced. The absorption cross-section of PS II will then be decreased (Mullineaux and Allen 1988), leading to a decrease in the turnover of PS II. This could occur, for example, in cyanobacterial cells with a high concentration of stored metabolites. Such cells would normally be in state 2, due to respiratory electron flow into the plastoquinone pool (Mullineaux and Allen 1986). The proportion of PS II functionally coupled to phycobilisomes would then be low (Mullineaux and Allen 1988) and as a result photodamage to PS II would be kept to a minimum.

Similarly, the presence of an exogenous electron donor will tend to reduce the plastoquinone pool, causing a transition to state 2 and thus ensuring that the majority of reducing equivalents are obtained from the electron donor rather than from PS II turnover. Some cyanobacteria are capable of utilising sulphide as an exogenous electron donor

to the photosynthetic electron transport chain. In the cyanobacterium *Oscillatoria limnetica*, the presence of sulphide strongly and reversibly inhibits PS II turnover (Belkin and Padan 1983). We suggest that this effect may be due to the induction of a state 2 transition.

The primary function of state transitions in cyanobacteria may therefore be to prevent photo-damage to PS II sustained during unproductive PS II turnover rather than to redistribute excitation energy between PS II and PS I.

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