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Control of excitation energy distribution in cyanobacteria: sensitivity to uncouplers and ATP synthase inhibitors

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State 1/State 2 transitions in 3-(3,4-dichlorophenyl)-1-1′-dimethylurea (DCMU)-poisoned *Anacystis nidulans* cells were monitored using modulated fluorescence techniques. Measurements of the sensitivity of these transitions to uncouplers and ATP synthase inhibitors indicated that much higher concentrations of such agents were required to inhibit the fluorescence changes than are required to inhibit non-cyclic electron transport in unpoisoned cells. Measurements of the intensity dependence of the light-driven fluorescence changes in the presence of uncouplers and ATP synthase inhibitors suggest that their inhibitory effect is due to a decreased ability of PS I to extract electrons from plastoquinone associated with the inhibition of non-cyclic electron transport. These results suggest that State 1/State 2 changes in cyanobacteria are under redox control. Measurements of cell ATP levels are shown to be consistent with, but not necessarily diagnostic of, the involvement of a redox-sensitive kinase system capable of phosphorylating key pigment protein complexes in this control system.

Introduction

Cyanobacteria show light-dependent changes in the distribution of excitation energy between Photosystem I (PS I) and Photosystem II (PS II) similar to those originally reported by Bonaventura and Myers [1] for green and by Murata [2] for red algae. In all these systems, exposure to light that preferentially stimulates PS I activity results in a transition to a state (State 1) in which the photosynthetic activity of PS II photoprocesses is increased. Conversely, exposure to light that preferentially stimulates PS II activity results in a state (State 2) in which the efficiency of PS II photoprocesses is decreased. These changes are thought to redress imbalances in the fraction of absorbed light delivered to the reaction centres of the two photosystems and hence to optimise the overall efficiency of photosynthesis. For a recent review of the literature relating to State 1/State 2 transitions in algae and higher plants, see Ref. 3.

In the case of higher plants and green algae, these transitions are widely belived to be associated with the phosphorylation of light-harvesting chlorophyll *a/b* protein (LHC II) [4–7]. Any initial imbalance between the photosystems in favour of PS II results in the preferential reduction of the plastoquinone pool lying between PS II and PS I. This leads to the activation of a kinase system, possibly associated with the cytochrome b_6/f complex [8], that phosphorylates LHC II bringing about changes in the organisation of the light-harvesting apparatus that result in a more equal distribution of excitation energy between the two photosystems. Imbalances in the distribution of excitation energy in favour of PS I, in contrast, lead to an inactivation of the kinase system and a dephosphorylation of LHC II. Algal systems are normally found to relax to a state similar, or identical, to State 2 in the dark [9–13]. The similarity of excitation energy distribution in State 2-adapted and dark-adapted cells has been explained in terms of a reduction of their plastoquinone pool, and hence an activation of their kinase system, by electron transport through the chlororespiratory pathway [14,15] identified by Bennoun [16].

Exposure of cyanobacteria, which lack LHC II, to light of different wavelengths leads to similar changes in the distribution of excitation energy between PS I and PS II. These changes can be monitored by measurements of changes in low temperature (77 K) fluores-

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCCD, *N,N′*-dicyclohexyl carbodiimide; DCMU, 3-(3,4-dichlorophenyl)-1,1′-dimethylurea; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; LHC II, light-harvesting chlorophyll *a/b* protein; PS I, Photosystem I; PS II, Photosystem II; TBT, tributyl-*n*-tin chloride.

cence emission [17,18], by light-dependent changes in the room-temperature fluorescence emission of pigments associated with PS II in DCMU-poisoned algae [18–25] or by photoacoustic methods [26]. Two mechanisms have been proposed to account for the control of excitation energy distribution in cyanobacteria. Allen and his co-workers proposed that they contain pigment-protein complexes that play a role analogous to that postulated for LHC II in higher plants and green algae [27–29]. Biggins and his co-workers [30], however, have put forward an alternative model for State 1/State 2 adaptation in red algae and cyanobacteria based on the idea that light preferentially absorbed by PS I results in an over-stimulation of cyclic electron transport. They suggested that proton fluxes associated with such transport lead to localised electrical changes in the thylakoid membrane resulting in a re-organisation of the membrane that decreases the efficiency of spillover of excess excitation energy from PS II to PS I.

It is generally accepted that the respiratory and photosynthetic electron transport chains of cyanobacteria intersect at the level of plastoquinone as illustrated in Fig. 1 (see reviews in Refs. 31–33 and references therein). Measurements of rates of cytochrome *f* oxidation [34,35], light-dependent inhibition of the release of $^{14}CO_2$ from pre-labelled cells [36], inhibition of respiratory electron flow [25,37] and Mehler activity [24] all suggest that PS I and the terminal oxidase for the respiratory chain are in direct competition for respiratory electrons flowing through the plastoquinone pool. Mullineaux and Allen [24] have presented evidence, based on the comparison of State 1/State 2 changes in starved and unstarved *Synechococcus* 6301 cells, suggesting that respiratory electron flow influences state transitions by altering the redox state of the plastoquinone pool. Their results suggest that respiratory electron flow activates a redox-sensitive kinase, keeping the cyanobacteria in State 2 in the dark, in a similar way to that postulated for chlororespiration in green algae. A discussion of the similarities between State 1/State 2 transitions in higher plant and cyanobacterial systems, in terms of intersecting electron transport chains, can be found in the review of Williams and Allen [3].

Dominy and Williams [25] reported that the light-dependent inhibition of respiratory electron flow in DCMU-poisoned *Synechococcus* 6301 (*Anacystis nidulans*) shows a similar intensity dependence to the light-induced increase in fluorescence associated with State 1 transitions in such cells. This observation supports the idea that such transitions are controlled by the redox state of photosynthetic electron transport chain intermediates. In this paper, we report measurements of the effect of uncouplers and ATP synthase inhibitors on the light-driven fluorescence changes seen in DCMU-poisoned *Synechococcus* that lend further support to this view.

Materials and Methods

Synechococcus leopolensis ((A. nidulans) UTEX 625, Collection of Algae, University of Texas, Austin) was cultured at 40 °C in Kratz and Myer's medium C [38], supplemented by the addition of 80 mg $\cdot l^{-1}$ of $Fe(SO_4) \cdot 7H_2O$, bubbled with 5% CO_2. The cell suspensions used for fluorescence measurements were normally grown under an incident light intensity of 80 μmol $\cdot m^{-2} \cdot s^{-1}$ (PAR). Cells were also grown under 200 μmol $\cdot m^{-2} \cdot s^{-1}$ (PAR) (high-light conditions) or 40 μmol $\cdot m^{-2} \cdot s^{-1}$ (PAR) (low-light conditions) when required. Cells were harvested in the early log-phase. The absorbance at 680 nm of test samples was normally in the range 0.2–0.3.

Fluorescence measurements were made using a Perkin-Elmer MPF-44A spectrofluorimeter modified by the incorporation of a stirrer and a fibre-optic to deliver a high-intensity actinic beam to the sample. The modulated measuring beam of the intrument was used to

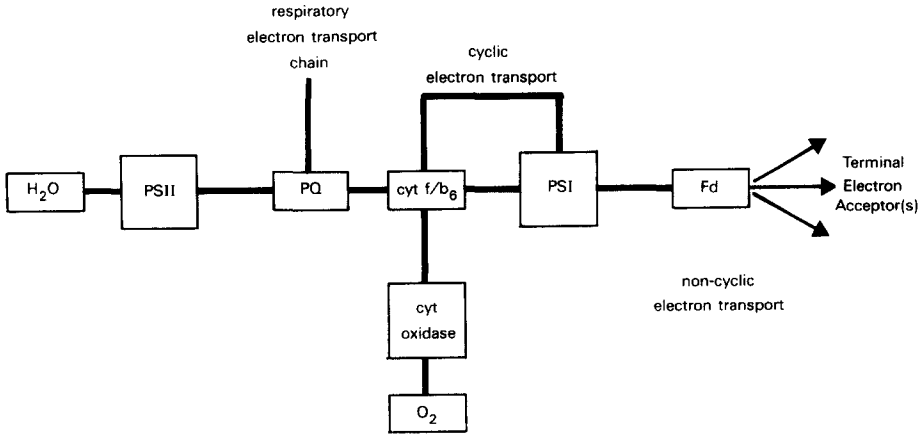

Fig. 1. Diagram illustrating the intersection of the respiratory and photosynthetic electron transport chains of cyanobacteria.

monitor sample fluorescence and the non-modulated actinic beam, isolated from a 250 watt quartz-halogen lamp using a combination of Schott broad-band blue glass filters, was used to drive State 1/State 2 changes. The intensity and wavelength of the measuring beam was 5.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 570 nm and, unless otherwise stated, the intensity of the actinic beam was 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Measuring and actinic beam intensities were reduced when required by the use of Balzer neutral-density filters and light intensities were measured using a Li-Cor (Lincoln, NE, U.S.A.) LI-190SB Quantum Sensor.

Photosynthetic oxygen evolution rates were measured using a Hansatech (U.K.) oxygen electrode. Samples were normally pre-equilibrated in the electrode chamber for 30 min prior to measurement so as to reduce problems associated with the build-up to high metabolite levels and/or high CO_2 levels. Measurements of oxygen evolution were performed using the same actinic light conditions as employed in the fluorescence measurements.

Cell ATP levels were estimated using the method of Lundin and Thore [39]. Cell suspensions were grown under 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to an absorbance of 0.3 at 680 nm. The cells were then pre-incubated in their culture tubes at 40°C under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ illumination for 90 min to stabilise their ATP levels at the higher light intensity. Small samples (0.5 ml) were withdrawn at intervals over a further period of 20 min for the determination of the basal ATP level of unpoisoned cells. At the end of this period, the cultures were divided into two 125 ml stock suspensions. DCMU (40 μM) was added to one of the suspensions and an appropriate amount of either the uncoupler CCCP or ATP synthase inhibitor DCCD was added to both suspensions. Samples of the two stock suspensions were removed for ATP analysis at fixed intervals. The cells were kept in light with air/CO_2 bubbling throughout the measuring period. Following their removal, the samples for analysis were immediately injected into 1-ml aliquots of ice-cold 0.56 M trichloroacetic acid (TCA)/5 mM EDTA. After a period of 20–30 min, the samples were washed three times with 4 ml vols. of diethyl ether to remove the TCA. Final traces of ether were removed by bubbling the samples with water-saturated air. The samples were then adjusted to pH 7.0 using a minimal volume of potassium hydroxide. Their ATP content was measured using 1243-200 firefly luciferin/luciferase ATP measuring reagent (LKB-Wallac) and a LKB-Wallac 1250 luminometer. An internal standard of 0.66 μM ATP was used to calibrate each measurement.

Results

Measurement of State 1 / State 2 transitions

State 1/State 2 transitions in algal cells are conveniently monitored by measuring changes in the fluorescence yield of dark-adapted cells illuminated in the presence of DCMU to block PS II activity [10,25]. The fluorescence yield of the cells is monitored using a relatively low intensity modulated measuring beam that is sufficiently intense to keep all PS II traps closed but too weak to influence the state of the cells. Under these conditions the cells remain in State 2. Exposure to a higher intensity, non-modulated actinic beam drives the cells to State 1. The detection system is arranged so that the unmodulated component of fluorescence emission, stimulated by the actinic beam, is rejected. Changes in the quantum yield of fluorescence associated with state transitions are, therefore, directly reflected in changes in the intensity of the modulated component of emission associated with the measuring beam. Measurements at lower temperatures, which result in marked decreases in the rate of the light-induced fluorescence increases [25], and ability of DBMIB to abolish these transitions (results not shown), confirm that these transitions reflect

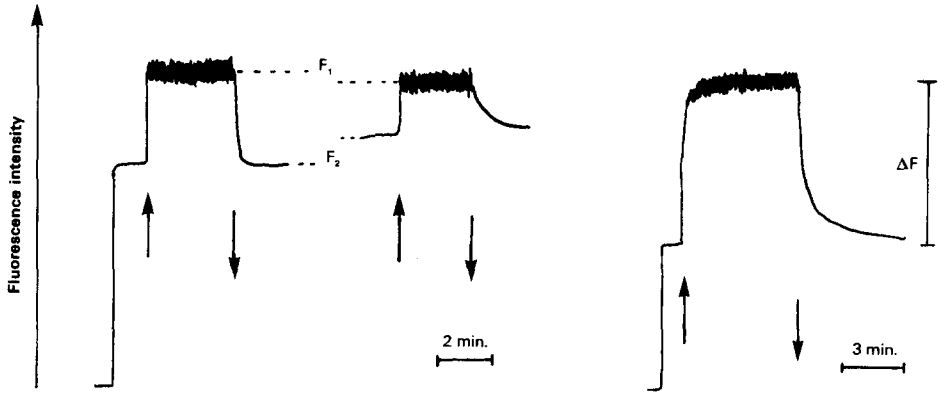

Fig. 2. Typical traces illustrating the light-driven fluorescence changes seen in *Synechococcus* cells poisoned with DCMU (40 μM). (a) Fresh sample of high-light grown cells, (b) same sample cycled from State 2 (3 min) to State 1 (3 min) for 90 min, (c) fresh sample of low-light grown cells. The arrows indicate the switching on and off of the actinic beam. The fluorescence yields of the cells in State 1 and State 2 are F_1 and F_2, respectively, and the light-driven fluorescence increase is given by ΔF.

secondary adaptation to the actinic light and are not due to changes in the redox state of PS II reaction centres.

Typical traces obtained for DCMU-poisoned *Synechococcus* cells are shown in Fig. 2. The relative increase in fluorescence $\Delta F/F_2$ (where $\Delta F = F_1 - F_2$ and F_1 and F_2 are the fluorescence yields in State 1 and State 2, respectively) varies with the intensity of the actinic light. Under saturating light conditions, the increase is normally between about 0.4 and 1.0. The precise value of $\Delta F/F_2$ varies markedly with the history of the cells. Rapidly growing cells, grown at low cell densities under high-light conditions, tend to give traces of the type shown in Fig. 2a that are characterised by relatively low $\Delta F/F_2$ values (0.4–0.6) and show very rapid kinetics. Slowly growing cells, grown under low-light or at high cell densities, tend to show higher $\Delta F/F_2$ values (0.6–1.0) and slower kinetics of the type shown in Fig. 2c. If the cells giving rise to the signal shown in Fig. 2a are continuously cycled between State 1 and State, 2, the rate and extent of the changes slowly decreases with time (see Fig. 2b). Similar effects are seen if the cells are kept in the dark for 2–3 h. The changes seen in the continuously cycled cells resemble those reported by Mullineaux and Allen [24] for starved cells. They attributed the changes seen in their study to the depletion of respiratory metabolites resulting in a decreased flow of respiratory electrons into the plastoquinone pool (cf. Fig. 1). They argued that under these conditions, the activity of the redox-sensitive kinase that normally maintains the cells in State 2 in the dark is lowered and the cells are in a state closer to State 1. The differences in $\Delta F/F_2$ seen in cells grown under different conditions in the present study are probably of similar origin.

Measurements using uncouplers and ATP synthase inhibitors

In the presence of DCMU, a protonmotive force can be generated in the dark by respiratory electrons flowing to the thylakoid-bound cytochrome oxidase. In the light, it could, in principle, be generated either by the flow of these electrons to the oxidase or PS I, or by cyclic electron transport around PS I. Addition of uncouplers would be expected to depolarise any protonmotive force generated by such mechanisms, while addition of ATP synthase inhibitors would be expected to oppose its dissipation. In terms of the model proposed by Biggins et al. [30], in which the transition from State 2 to State 1 is thought to result from a protonmotive force generated by cyclic electron transport, the presence of uncouplers would thus be expected to abolish State 1 adaptation while the presence of ATP synthase inhibitors might be expected to inhibit the restoration of State 2. Prediction of the effects of such agents in the case of the pigment-protein phosphorylation model is more difficult as the state of the cells is dependent both on the presence of ATP to phosphorylate the pigment-protein complexes and the redox state of the electron transport intermediates controlling the kinase system leading to such phosphorylation.

In practice, the effects of uncouplers and ATP synthase inhibitors on State 1/State 2 transitions in DCMU-poisoned cyanobacteria were found to be extremely complex. Typical traces for nigericin and DCCD are shown in Figs. 3 and 4, respectively. Two distinct

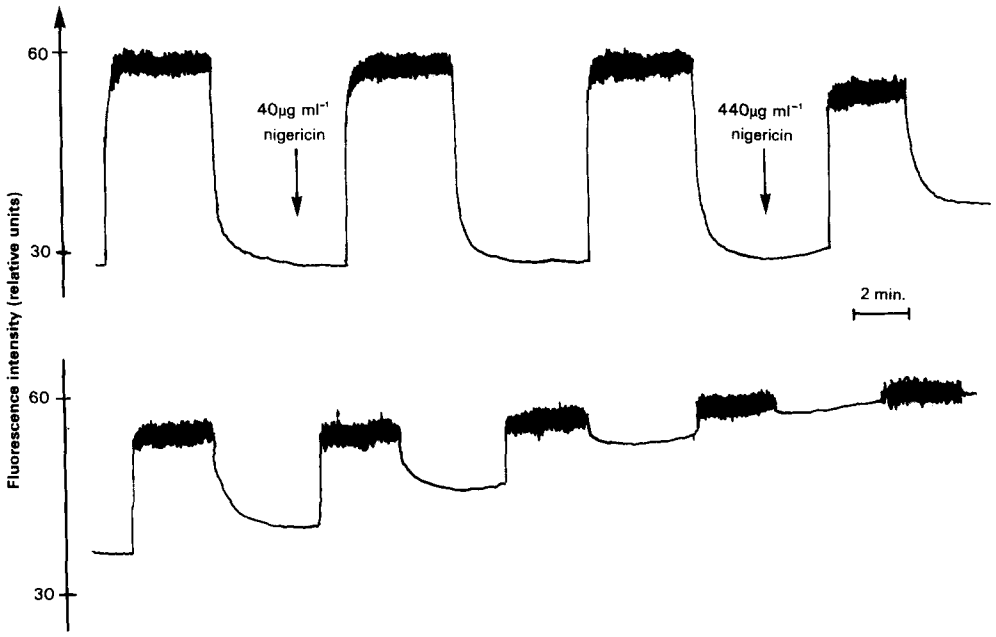

Fig. 3. Typical trace of the light-driven fluorescence changes seen in DCMU-poisoned *Synechococcus* following the addition of nigericin.

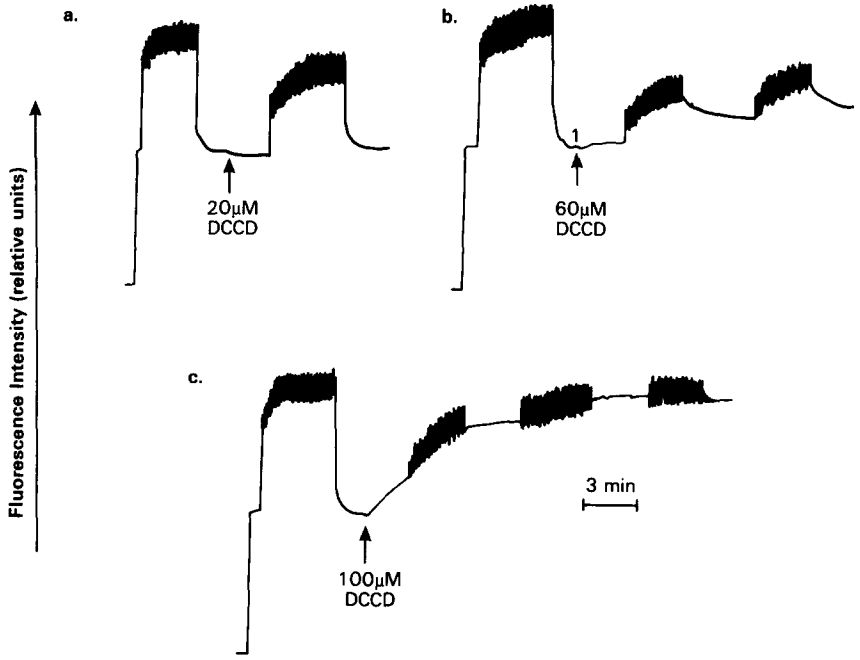

Fig. 4. Typical traces of the light-driven fluorescence changes seen in DCMU-poisoned *Synechococcus* following the addition of DCCD.

effects can be identified. The first, and most immediate, is a lowering of the value of F_1. This effect appears to predominate at low uncoupler/inhibitor concentrations. The second effect takes place on a slower timescale and involves a rise in the value of F_2. The rate of the rise in F_2 increases with uncoupler/inhibitor concentration. It is particularly marked for the inhibitors DCCD and TBT, but is also seen at higher concentrations of nigericin and CCCP. The rate of rise in F_2 appears to be light-independent.

In order to separate out the immediate effects of the different treatments from their longer-term effects. The concentration dependencies of the initial lowering of F_1, as reflected in decreases in the value of $\Delta F/F_2$, were determined using a fresh sample for each measurement. The values of $\Delta F/F_2$ used in the plots were then calculated from the first cycle following the addition of the uncoupler or inhibitor. In the case of TBT, which is a lipophilic cation, the rate of uptake by *Synechococcus* is strongly influenced by light and a different approach had to be adopted. TBT was added in small aliquots making fluorescence measurements between each addition. Typical plots for the uncouplers CCCP and nigericin and for the inhibitors DCCD and TBT are shown in Figs. 5 and 6, respectively. Measurements showing the sensitivity of oxygen evolution to these agents in DCMU-free cells under the same light conditions are included in the plots for comparison. The oxygen evolution values correspond to net increases with respect to dark control levels. In all cases, it was necessary to use uncoupler/inhibitor concentrations sufficient to bring about complete, or near complete,

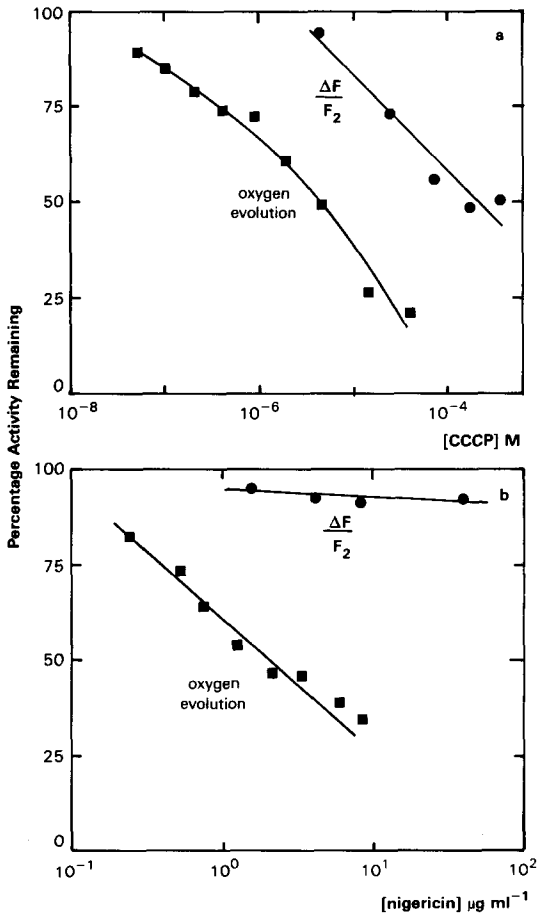

Fig. 5. Plots illustrating the dependence of the light-driven increase in the fluorescence yield, $\Delta F/F_2$, associated with State 1/State 2 transitions in DCMU-poisoned *Synechococcus* (●), and the inhibition of oxygen evolution in DCMU-free cells (■), on (a) CCCP and (b) nigericin concentration.

elimination of oxygen evolution before obtaining measurable changes in fluorescence.

Similar results (not shown) were obtained using valinomycin and nigericin plus valinomycin. Some experimental difficulties were, however, encountered when the uncoupler methylamine and the inhibitor phloridzin were used. The presence of high concentrations of methylamine was found to lead to appreciable photo-bleaching of the cells. Addition of phloridzin led to an inhibition of oxygen evolution but no changes in the fluorescence of DCMU-poisoned cells were seen using concentrations up to its solubility limit.

Earlier studies have shown a strong dependence of the light-driven fluorescence changes seen in DCMU-poisoned *Synchococcus* on the intensity of the actinic light [25]. Experiments were, therefore, carried out to check that the changes shown in Figs. 3 and 4 were true inhibitions and not merely reflections of changes in the sensitivity of the samples to light. Additions of uncouplers and inhibitors, both led to marked changes in the intensity dependence of the fluorescence changes

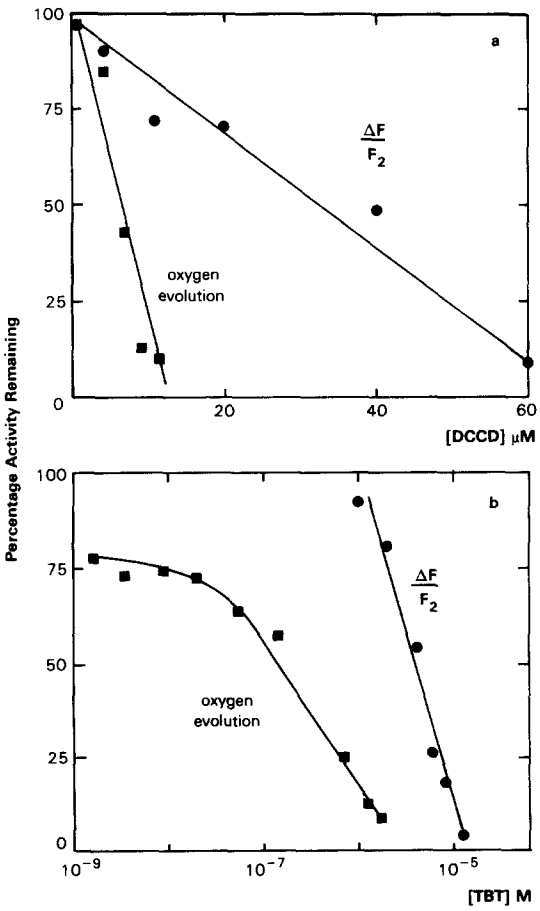

Fig. 6. Plots illustrating the dependence of the light-driven increase in the fluorescence yield, $\Delta F/F_2$, associated with State 1/State 2 transitions in DCMU-poisoned *Synechococcus* (●), and the inhibition of oxygen evolution in DCMU-free cells (■), on (a) DCCD and (b) TBT concentration.

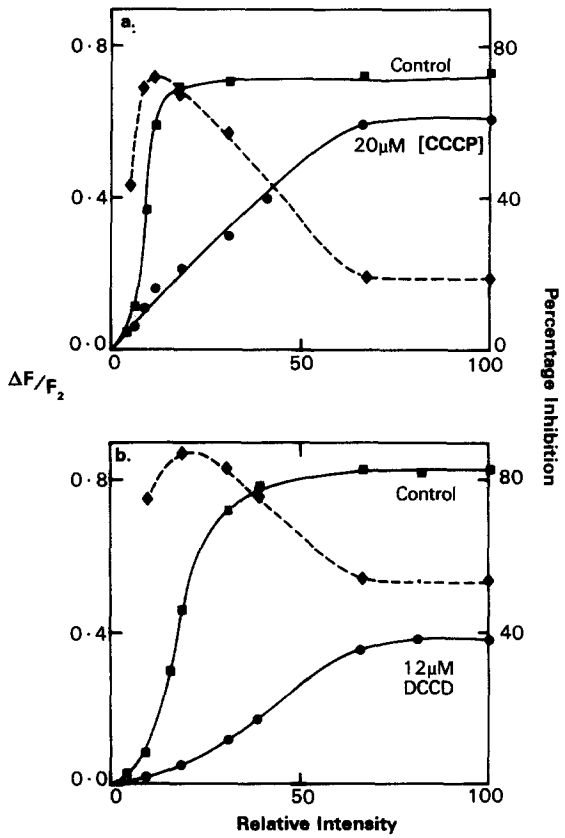

Fig. 7. Plots of the light-driven fluorescence increase ($\Delta F/F_2$) seen in DCMU-poisoned *Synechococcus* cells as a function of actinic light intensity (a) for control cells (■) and cells treated with 20 μM CCCP (●), (b) for control cells (■) and cells treated with 12 μM DCCD (●). The percentage inhibition of the light-driven fluorescence increases is indicated by the dashed line.

accompanying state transitions. Typical plots of the intensity dependence of $\Delta F/F_2$, in the presence and absence of uncouplers, as exemplified by CCCP, and ATP synthase inhibitors, as exemplified by DCCD, are presented in Fig. 7, a and b, respectively. In all cases, the extent of inhibition of the light-driven fluorescence changes shows a strong dependence on actinic light intensity, particularly in the low-intensity range. The State 1/State 2 transitions reported in this paper were all made at saturating intensities but the intensity measurements emphasise the care that has to be taken to avoid artefacts associated with changes in intensity dependence in the measurement of such transitions.

The experiments described above suggest that despite their very different modes of action, the effects of adding uncouplers and ATP synthase inhibitors to DCMU-poisoned algae are surprisingly similar. Experiments were, therefore, carried out to determine whether the effects of uncouplers and ATP synthase inhibitors were additive or antagonistic. Typical fluorescence traces for cells treated first with DCCD and then nigericin are shown in Fig. 8. Addition of nigericin to cells pre-treated with DCCD leads to a slow increase in the value of F_1

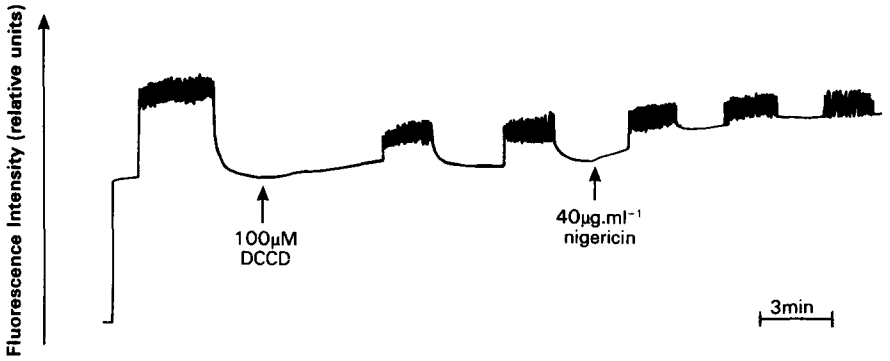

Fig. 8. Trace illustrating the effects on the fluorescence yield changes associated with State 1/State 2 transitions in DCMU-poisoned *Synechococcus* treated first with 100 μM DCCD and then with 40 $\mu g \cdot ml^{-1}$ nigericin.

and a rather faster increase in the value of F_2. These changes are similar to, but rather more rapid than, the changes seen on adding nigericin alone (see Fig. 2) suggesting that the effects of the uncoupler and inhibitor are additive. Similar results are obtained if TBT is used, providing the experimental protocol makes allowance for the initial light-dependent uptake of TBT (results not shown).

ATP measurements

The effects of uncouplers and ATP synthase inhibitors on cell ATP levels were measured in order to check whether their inhibitory effects on State 1/State 2 transitions were likely to be a reflection of ATP depletion. Samples of cells (250 ml) were pre-incubated in the light so as to stabilise their ATP levels. The samples were then divided into two stock suspensions. DCMU (40 μM) was added to one suspension to block PS II-mediated non-cyclic electron transport. Measurements of the effects of the uncoupler CCCP and the ATP synthase inhibitor DCCD on cell ATP levels of the two suspensions were made using the bioluminescence assay described in the methods section. The results of these measurements are presented in Figs. 9 and 10, respectively. They show that 10 μM CCCP, which is sufficient to inhibit oxygen evolution almost completely, but has little effect on the fluorescence yield changes associated with State 1/State 2 changes, has very little effect on the level of ATP in the cells. In contrast addition of 100 μM CCCP, which leads to an appreciable inhibition of the fluorescence changes (Fig. 5), leads to a very marked reduction in ATP levels.

In the case of DCCD, the situation is a little more complicated. Addition of 10 μM DCCD, which is sufficient to inhibit photosynthetic oxygen evolution, leads to a marked decrease in ATP levels over a period of about 20 min. Addition of 100 μM DCCD also leads to a marked reduction in ATP levels, but in this case the reduction is completed within 2–3 min. This behaviour, as discussed below, shows interesting parallels with the

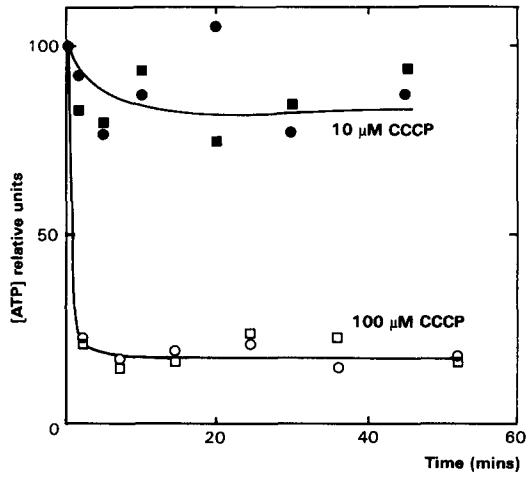

Fig. 9. Plot showing the ATP levels in extracts of cells treated with 10 μM or 100 μM CCCP as a function of incubation time before extraction. Measurements were made in the presence (●,○) and absence (■,□) of 40 μM DCMU. All values are normalised to those obtained for extracts of untreated cells.

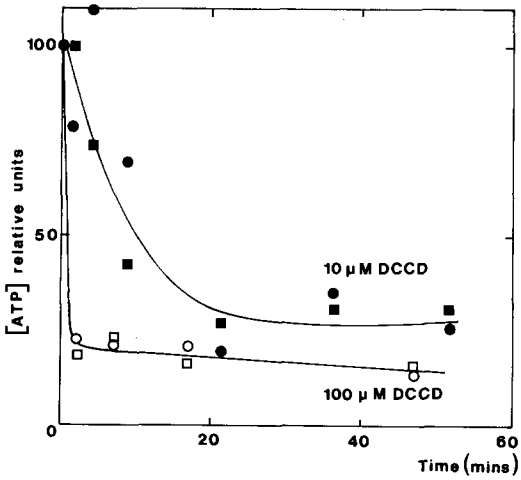

Fig. 10. Plot showing the ATP levels in extracts of cells treated with 10 μM or 100 μM DCCD as a function of incubation time before extraction. Measurements were made in the presence (●,○) and absence (■,□) of 40 μM DCMU. All values are normalised to those obtained for extracts of untreated cells.

fluorescence changes associated with State 1/State 2 changes observed for DCCD-treated cells (Fig. 4).

The presence or absence of DCMU seemed to have little or no effect on the ATP levels found in cells treated with uncouplers or ATP synthase inhibitors suggesting that, in the short term, DCMU itself has no significant effect on cell ATP levels. However, even with the highest concentrations of CCCP and DCCD, signals corresponding to ATP levels approx. 15–20% of the original levels were still detected. It is not clear whether they correspond to a true basal level of ATP, possibly reflecting substrate-level phosphorylation activity, or background interference.

Discussion

In cyanobacteria, it is generally agreed that State 2, the low fluorescence state, arises when there is a transfer of excitation from PS II to PS I, while State 1, the high fluorescence state, arises when this transfer is inhibited [3,30,43]. It is becoming increasingly clear that this transfer is mediated mainly through the chlorophyll-protein complexes of PS II. This is indicated by the demonstration of State 1/State 2 transitions produced by direct excitation of the chlorophyll *a* components of PS II of *Synechococcus* 6301 [25], and by the occurrence of normal state adaptations in a mutant of *Synechococcus* 7002 that does not assemble functional phycobilisomes [43].

There is, however, still considerable controversy regarding both the mechanisms controlling excitation energy transfer and the nature of the actual transfer process. One model suggests that the cells are in State 2 when the local protonmotive force across the thylakoid membrane generated by cyclic electron transport around PS I is small (e.g., when light is predominantly absorbed in PS II or the cells are in the dark), but in State 1 under conditions when this force is large (e.g., when light is predominantly absorbed in PS I or by cells poisoned with DCMU) [30]. The opposing view is that state adaptations in cyanobacteria are dependent upon the phosphorylation of polypeptides associated with PS II by a redox-sensitive kinase [27]. In both cases, for the reasons set out earlier, state transitions would be expected to be sensitive to uncouplers and ATP synthase inhibitors.

Considerable confusion exists in the literature regarding the effects of uncouplers and ATP synthase inhibitors on the light-driven fluorescence increases associated with State 1 adaptation seen in DCMU-poisoned cyanobacteria. It is generally recognised that such transitions are inhibited by uncouplers [18,20,40]. There is less agreement in the case of the ATP synthase inhibitors. While it is agreed that state changes are insensitive to phlorzin [18,20,40], they have been reported to show a limited sensitivity to Dio-9 [40] and conflicting reports have appeared concerning their sensitivity to DCCD (cf. Refs. 18 and 40). Much of this confusion can be attributed to the use of inappropriate concentrations of these inhibitors. It has been tacitly assumed that they should be used at similar concentrations to those normally used in chloroplast photophosphorylation and oxygen evolution studies. In practice, it is necessary, as illustrated by the results presented in Figs. 5 and 6, to use much higher concentrations than those required to inhibit non-cyclic electron transport in DCMU-free cells. Under these conditions, both uncouplers and ATP synthase inhibitors, with the exception of phloridzin which is too insoluble to allow the use of sufficiently high concentrations, have marked effects on State 1/State 2 transitions.

The cyclic electron transport model of Biggins et al. [30] correctly predicts that addition of uncouplers/ionophores, which depolarise the thylakoid membrane, should tend to abolish the light-driven fluorescence increase normally seen on exposure of DCMU-poisoned cells to the actinic beam (Figs. 3 and 5). It does not explain, however, why the addition of ATP synthase inhibitors, which would be expected to hyperpolarise this membrane, also appear to inhibit this transition (Figs. 4 and 6). Nor does it offer any obvious explanation of the longer-term changes in fluorescence yield associated with the addition of uncouplers or inhibitors shown in Figs. 3 and 4.

These observations can, however, be relatively easily explained in terms of the redox control model. In terms of this model, the photosynthetic electron transport components lying between PS II and PS I are thought to be maintained in a reduced state in the dark by respiratory electron transport, via a thylakoid-bound NAD(P)H dehydrogenase, as illustrated in Fig. 1. The cells are thus held in State 2 in the dark. Exposure to light in the presence of DCMU results in the oxidation of the photosynthetic electron transport chain by PS I-mediated non-cyclic electron transport. Considerable evidence, as explained in the Introduction, exists for the flow of electrons from respiratory metabolites through the photosynthetic electron transport chain to PS I in cyanobacteria under these conditions. The initial lowering of the F_1 level of fluorescence by uncouplers and ATP synthase inhibitors can thus be explained either by an inhibition of the PS I-mediated oxidation of the cytochrome b_6/f complex, if a purely redox mechanism is operating, and/or by a lowering of cellular ATP levels, if pigment-protein phosphorylation is involved.

Measurements of the intensity dependence of the light-driven transition from State 2 to State 1 in the presence of uncouplers and ATP synthase inhibitors (Fig. 7) suggest that they reduce the ability of PS I to extract electrons from the plastoquinone pool. The basis of this inhibition is still uncertain, as the nature of the terminal electron acceptor operating in DCMU-poi-

soned cells is not known. It is clear, however, that electron acceptors other than oxygen are involved as State 1/State 2 change in DCMU-poisoned cells are largely unaffected by anaerobosis [25]. Most of the possible alternative candidates, such as NO_3^-, SO_4^{2-}, and CO_2 involve energy-dependent pathways and are, hence, dependent on continued ATP synthesis.

The pigment-protein phosphorylation model predicts that the pigment-protein complexes should be phosphorylated in the low fluorescence state. In terms of this model, the observation that uncouplers and ATP synthase inhibitors tend to lower the fluorescence yield of the illuminated cells might seem anomalous. However, in agreement with earlier studies [41,42], we find that ATP levels in cyanobacteria are relatively insensitive to the addition of levels of uncouplers/inhibitors. This suggests that in the low concentration range, at least, there would probably still be sufficient ATP available to maintain any pigment-protein complexes in the phosphorylated state as long as the redox-sensitive kinase remains switched on.

Addition of high concentrations of uncouplers and ATP synthase inhibitors, leads to an initial fall in the light-driven fluorescence increase followed by a slower light-independent rise to levels typical of State 1 (Figs. 2 and 3). Marked falls in the level of ATP in the cells also occur under these conditions (Figs. 9 and 10), suggesting that the availability of ATP rather than the redox state of the cytochrome b_6/f complex may be the limiting factor. The light-independent rise in fluorescence can be explained on this basis by a dephosphorylation of the pigment-protein complexes which would be independent of the redox state of this complex under these conditions. This view is consistent with the increasing inability of the inhibited cells to relax into State 2 in the dark (Figs. 3 and 4). The fact that the rate of increase in the value of F_2 in DCCD-preated cells appears to mirror the rate of fall in cells ATP levels may also be significant. At low DCCD concentrations, the rise in the value of F_2 (Fig. 3) and the rate of decrease in ATP (Fig. 10) are both relatively slow, while at high concentrations both parameters exhibit much more rapid changes.

In conclusion, our results lend strong support to the idea that state changes in cyanobacteria are under redox control. They are also consistent with the idea that this control is exerted by a redox-sensitive kinase. A final resolution on this latter point must, however, await direct measurements of pigment-protein phosphorylation under in vivo conditions.

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