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EXCITATION-ENERGY DISTRIBUTION IN GREEN ALGAE

THE EXISTENCE OF TWO INDEPENDENT LIGHT-DRIVEN CONTROL MECHANISMS

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Three distinct states can be identified for cells of the green alga *Chlorella vulgaris*; State 1 and State 2 obtained by preillumination in far-red and red light, respectively, and the dark state obtained by dark-adaptation. Addition of the inhibitor DCMU to algal cells leads to an initial rapid increase in chlorophyll-*a* fluorescence reflecting the closure of Photosystem II traps. This, in the case of dark and state-2-adapted algae is followed by a slow light-dependent increase to a fluorescence yield typical of State-1-adapted cells. Measurements of low temperature (77 K) emission spectra indicate that the low fluorescence yields of dark and State-2-adapted algae reflect similar balances in excitation-energy distribution between the two photosystems. In both cases, the balance favours PS I and the slow fluorescence increase seen in the poisoned algae reflects a redressing of this balance in favour of PS II. The low fluorescence yield of State-2-adapted algae is thought to be associated with the phosphorylation of chlorophyll *a/b* light-harvesting protein (Biochim. Biophys. Acta (1983) 724, 94–103). Measurements of the uncoupler and ATPase sensitivity of the light-dependent increases seen in DCMU-poisoned cells indicate that the low fluorescence yield of dark-adapted algae is of different origin. Evidence is presented showing that the light-driven changes in excitation-energy distribution seen in green algae involve two distinct processes; a low-intensity, wavelenght-independent change reflecting simple light/dark changes and a higher intensity, wavelength-dependent change reflecting State 1/State 2 adaptation. The former changes appear to be associated with changes in the local ionic environment within the algal chloroplast, whilst the latter appear to reflect changes in the phosphorylation state of chlorophyll *a/b* light-harvesting protein.

Introduction

The occurrence of light-driven changes in the distribution of excitation energy between Photosystem I (PS I) and Photosystem II (PS II) was first reported by Bonaventura and Myers [1] and Murata [2]. They showed that algal cells illuminated by light preferentially absorbed in PS I undergo a transition to a state (State 1) characterised by a high efficiency of PS II photoprocesses, whilst cells illuminated by light preferentially absorbed in PS II undergo a transition to a state (State 2) characterised by a high efficiency of PS I photoprocesses. Similar changes have since

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Abbreviations: PS I, Photosystem I; PS II, Photosystem II; LHC-II, chlorophyll *a/b* light-harvesting protein; DCMU, 3-(3′,4′ dichlorophenyl)-1,1′-dimethylurea; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N*′-dicyclohexylcarbodiimide.

been shown to occur in intact leaves of higher plants [3,4] and osmotically shocked chloroplasts [5]. They are thought to reflect the operation of a control mechanism that attempts to balance the relative inputs of the two photosystems. In the absence of such a system, preferential excitation of one or other of the two photosystems would result in a drop in the overall quantum efficiency of photosynthesis [6].

Higher plant chloroplasts contain kinase [7] and phosphatase [8] systems that can phosphorylate, or dephosphorylate, chlorophyll *a/b* light harvesting protein (LHC II), the major chlorophyll-protein complex of the PS II light-harvesting system. Equivalent systems are found in green algae. Parallel measurements of the phosphorylation of LHC II and fluorescence changes in higher plant [9] and algal chloroplasts [10], indicate that LHC II phosphorylation is associated with an increase in the fraction of incident light absorbed by, or transferred to, PS I. Allen et al. [11] and Horton et al. [9] have proposed that these changes are part of a feedback mechanism in which the imbalance between the photchemical activities of PS I and PS II is sensed via the redox state of the plastoquinone pool between the two photosystems. Overstimulation of PS II leads to a reduction of the plastoquinone pool. This, it is suggested, activates the kinase system resulting in a phosphorylation of LHC II and redistribution of excitation energy in favour of PS I, thus relieving the imbalance between the photsystems. Overstimulation of PS I, in contrast, leads to an oxidation of plastoquinone. Under these conditions, the kinase system is deactivated, and LHC II dephosphorylates leading to a redistribution of excitation energy in favour of PS II.

It is well documented in the literature that many of the effects associated with light-driven and LHC II phosphorylation-linked changes in excitation-energy distribution can be mimicked by changes in divalent cation levels in isolated chloroplasts [12]. The fluorescence changes, in this case, are ascribed to changes in thylakoid organisation associated with electrostatic shielding of negatively charged groups on the thylakoid surface [13]. In a recent paper [10], we provided evidence showing that State 1/State 2 changes in *Chlorella* cells are consistent with the predictions of the LHC-II phosphorylation model. In this paper, we show that a more complete description of the light-driven changes seen in intact cells is obtained if elements of the LHC-II phosphorylation and divalent cation models are combined into a single model.

Materials and Methods

Algae culture. Chlorella vulgaris 8k (high-temperature strain), obtained from the Cambridge Culture Collection, was grown in batch culture at 28°C under about 10000 lux in Knop's medium bubbled with 3% CO_2.

Fluorescence measurements. All fluorescence measurements were made using a spectrofluorimeter constructed in this laboratory [14]. The instrument was normally operated in an a.c. mode using a 62 Hz measurement beam ($\lambda = 440$ nm; intensity 27 $mW \cdot m^{-2}$) to excite chlorophyll *a* fluorescence detected at 685 nm. The instrument is fitted with two stronger d.c. lights, an actinic light source (ALS) used to ensure that all PS-II traps are closed, and thus that all fluorescence measurements are made at the F_M level, and a variable intensity light source (PLS) used for preillumination of the samples. The actinic beam was isolated from a 250 W tungsten-iodide lamp using a combination of Schott BG 11 and BG 38 broad-band filter and the red and far-red preilluminating beams were isolated from a 500 W tungsten filament lamp using 650 or either 702 or 706 nm Balzer (B40) interference filters. Balzer neutral-density filters were used to attentuate the intensity of these beams when required. The instrument can also be used in a d.c. mode using the actinic light source to provide an intense unmodulated excitation beam when required.

Room temperature fluorescence measurements were made on samples containing 2–4 μg Chl/ml, held in 10 mm fluorimeter cuvettes. Low temperature (77 K) measurements were made on samples containing approx. 15 μg Chl/ml sandwiched between two cover slips with a 0.5 mm Teflon spacer. Phycocyanin, isolated from *Anacystis nidulans*, was used as an internal standard to allow normalisation of the spectra [10,15].

Oxygen evolution measurements

The intensity dependence of oxygen evolution in *Chlorella* was measured using a Hansatech (U.K.) oxygen electrode.

Results

Steady-state fluorescence

The fluorescence yield of chlorophyll *a* from *Chlorella* cells at room tempreature is determined by three factors; the fraction of open PS II traps, the extent of energy-dependent quenching and the extent of State 1/State 2 adaptation. The relative importance of these three factors in determing the fluorescence yield of a given sample can be assessed by measurements of the fluorescence changes occurring following the addition of DCMU. Typical traces illustrating such changes are presented in Figs. 1 and 2.

Addition of DCMU leads to a rapid closure of PS II traps as Q, the primary electron acceptor of PS II, becomes reduced. This results in a rapid increase in fluorescence yield ($t_{1/2} < 1$ s) reflecting the relief of Q-dependent fluorescence quenching [16]. This initial rise, referred to as R_{fast} in Fig. 1, is followed by a rather slower rise, R_{slow}, with $t_{1/2} \simeq 10$ s, associated with the relief of energy-dependent quenching [17]. This latter type of quenching which is observed at relatively low incident-light intensities in isolated chloroplasts [18–23] is only seen at relatively high intensities in algal cells. The trace shown in Fig. 1 was measured using an incident intensity of 200 $W \cdot m^{-2}$ which is approx. 40% of that required to saturate photosynthesis in these cells. Under these conditions, energy-dependent quenching represented about 6% of the value of the maximal fluorescence yield. The decreased importance of energy-dependent quenching under in vivo conditions probably reflects a more efficient energy coupling between the light reactions and the Calvin cycle than that normally seen in isolated chloroplasts. No energy dependent quenching was observed at incident intensities below 30 $W \cdot m^{-2}$. Under these conditions addition of DCMU led to a rapid increase in fluorescence yield direct to its maximal value. All the measurements reported below were made at intensities well below this level, so as to avoid any possible complications associated with this type of quenching.

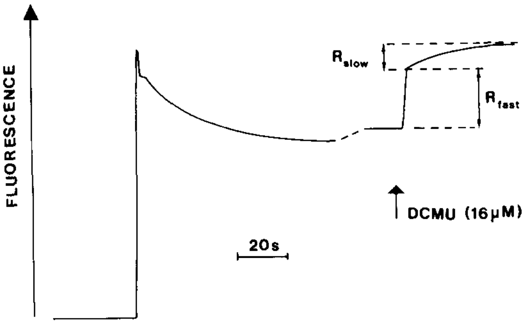

Fig. 1. Fluorescence yield changes occurring on poisoning *Chlorella* cells illuminated for 15 min with high intensity (200 $W \cdot m^{-2}$) broad band blue light. Addition of DCMU (16 μM) results in a biphasic fluorescence increase; R_{fast} reflecting relief of Q-dependent quenching and R_{slow} reflecting relief of energy-dependent quenching. The fluorimeter was operated in a d.c. mode using the actinic light beam (ALS) as the excitation source. Fluorescence emission was monitored at 685 nm.

The relatively rapid changes associated with the relief of Q-dependent and energy-dependent quenching are easily distinguished from those associated with State 1/State 2 changes which take place on a much slower time-scale. Typical traces illustrating such changes for algal samples preadapted to State 1 or State 2, by 20 min preillumination in far-red (702 nm) or red (650 nm) light respectively, or to the dark state by 20 min dark adaptation are presented in Fig. 2. Following the addition of DCMU, the PS-II traps of the samples close and the fluorescence yield rapidly increases to a maximal level, F_M, typical of the preadapted state of the sample. DCMU blocks PS-II activity and any further illumination, independent of wavelength, tends to lead to State 1 adaptation [18]. The slow light-dependent increases in fluorescence shown in Fig. 2 reflect transitions between the preadapted states characterised by F_{M_I}, $F_{M_{II}}$ and F_{M_D} (where the subscripts refer to State 1, State 2 and dark, respectively). The small increase in fluorescence yield (ΔF) seen for the samples preilluminated in far-red light probably reflects the fact that absorption even at these wavelengths is not entirely restricted to PS I and suggests that State 1 adaptation is not fully developed unless all PS II activity is suppressed. These slow light-dependent fluorescence increases are reversed in the dark. The decay of the fluorescence signal as the system relaxes from

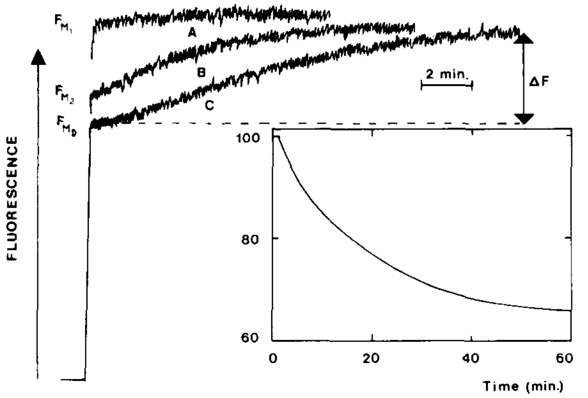

Fig. 2. Slow light-driven fluorescence increases occurring following poisoning of *Chlorella* with DCMU (6 μM). Cells were adapted to State 1 (A) or State 2 (B) by preillumination for 20 min in far-red light ($\lambda = 702$ nm; intensity 20 $W \cdot m^{-2}$) or red light ($\lambda = 650$ nm; intensity 20 $W \cdot m^{-2}$) or adapted to the dark state (C) for 20 min prior to poisoning. The fluorimeter was operated in an a.c. mode using a modulated measuring beam ($\lambda = 440$ nm; intensity 27 $mW \cdot m^{-2}$) too weak to drive State 1/State 2 changes together with a blue broad band d.c. actinic beam (intensity 69 $W \cdot m^{-2}$) to keep all PS-II traps closed. The inset shows the dark reversal of these changes, on removal of the actinic beam, measured in the presence of 0.5 mM NH_2OH to keep the PS-II traps closed, expressed as percentage reduction of maximal fluorescence yield. Fluorescence emission was monitored at 685 nm.

State 1 to the dark-adapted state can be monitored using a very low intensity measuring beam as long as hydroxylamine (0.5 mM) is added to prevent the reopening of closed PS II traps (see inset to Fig. 2). The half-time of this decay varies with the matabolic state of the algal cells but usually lies in the range 5–15 min.

Although dark and State-2-adapted algae are both characterised by relatively low values of F_M, the two states are clearly not identical. The light-dependent fluorescence increases seen for dark-adapted algae are both larger and appreciably slower than those seen for State-2-adapted algae (Fig. 2). Further evidence of differences between the two states is provided by the adaptation measurements presented in Fig. 3. Samples of algae initially preadapted to State 1 by a 15 min exposure to far-red light were then either exposed to red light or dark adapted for different periods. At the end of these periods, the value F_M was monitored by the addition of DCMU as described above. State-2 adaptation resulted in a fairly rapid ($t_{1/2} \simeq 1$ min) drop in F_M of about 17%. Dark adaptation, in contrast, involved a much slower ($t_{1/2} \simeq 7$ min) fall in value of F_M of up to about 35%. If following State 2 adaptation, the algae were put into the dark, they showed a further decrease in F_M to the level typical of the fully dark-adapted algae.

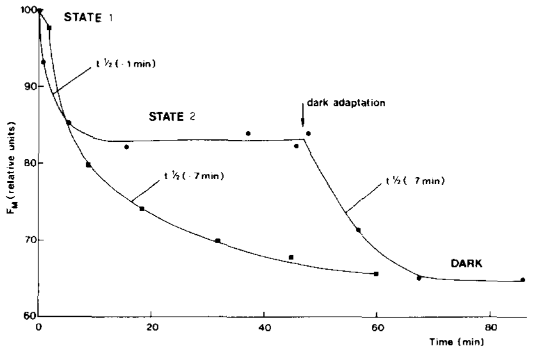

Fig. 3. Fluorescence yield changes associated with the State 2 and dark adaptation of State-1-preadapted algae. Chlorella cells were first adapted to State 1 by exposure to far-red light and then exposed to red light or dark adapted for different periods. The extent of the final adaptation to State 2 or dark was monitored by measuring F_M as described in the caption to Fig. 2.

The fluorescence yield of algal cells is known to decrease if they are allowed to become anaerobic [24]. Measurements of oxygen tension indicated that depletion of oxygen by cellular respiration during dark adaptation was not an important factor in these experiments. Indeed, similar measurements conducted using samples that were continuously aerated during adaptation yielded essentially identical results. It is thus clear that State 1, State 2 and the dark state represent three quite distinct states.

Low-temperature (77 K) emission spectra

The fact that the values of F_{M_D} and $F_{M_{II}}$ are both appreciably lower than F_{M_I} suggests that smaller fractions of the absorbed excitation energy are reaching PS II in dark and State-2-adapted algae than in samples adapted to State 1. In an earlier paper [10], we compared the low temperature emission spectra of State-1- and State-2-adapted *Chlorella* cells and showed that State-2 adaptation was associated with a redistribution of

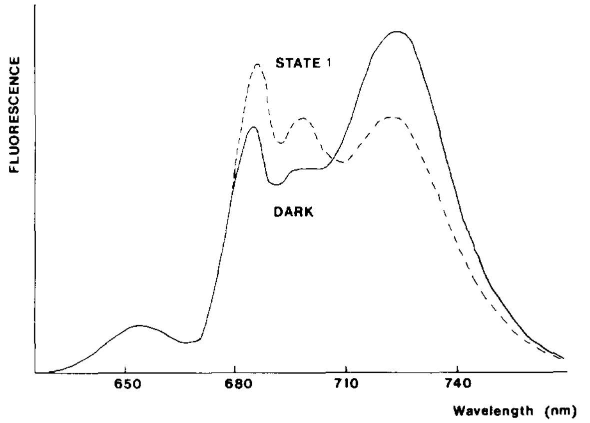

Fig. 4. Low-temperature (77 K) emission spectra of *Chlorella* cells adapted to State I and the dark state, as described in the caption to Fig. 2, directly prior to freezing. Spectra are normalised to the 655 nm peak of phycocyanin added as an internal standard.

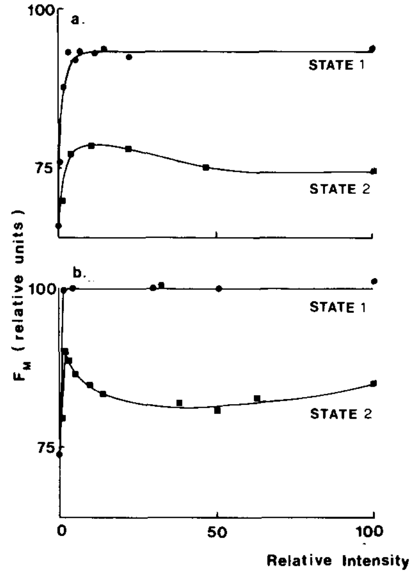

Fig. 5. Intensity dependence of State 1 and State 2 adaptation. *Chlorella* cells were first preadapted to the dark state. In (a) the cells were subsequently exposed for 15 min either to far-red light (●) or red light (■) to drive them to State 1 or State 2, respectively. In (b) they were exposed to red light, in the presence (●) or absence (■) of 16 μM DCMU. The values of F_M following exposure to different light intensities were monitored as described in the caption to Fig. 2. Maximum incident light intensities were 2.44 and 6.3 $W \cdot m^{-2}$ for the red and far-red light, respectively, used in (a), and 6.3 $W \cdot m^{-2}$ for the red light in (b).

excitation energy between the two photosystems in favour of PS I. Corresponding spectra for dark and State-1-adapted algae are shown in Fig. 4. As in the previous study, the spectra have been normalised using phycocyanin as an internal fluorescence standard. The two shorter wavelength peaks, at 685 and 695 nm are associated with PS II and the longer wavelength peak at about 710 nm with PS I [25]. Dark-adaptation leads to a lowering of the PS II-associated peaks and a corresponding increase in the height of the PS I-associated peak. These changes are directly analogous to those seen following State-2 adaptation [10], indicating that the two processes lead to similar changes in excitation-energy distribution. In both cases, the changes closely resemble those seen for samples of isolated chloroplasts in which LHC II has been phosphorylated by the addition of ATP [10,26] or magnesium has been omitted from the suspension medium [27].

Intensity dependence of State 1/State 2 adaptation

Measurements of the intensity dependence of State 1/State 2 adaptation of dark state algae are presented in Fig. 5. In these experiments, the algal samples were first dark adapted for 20 min and then exposed to different intensities of red or far-red light to adapt them to State 2 or State 1. The extent of this adaptation was then monitored for the different samples by measurements of the value of F_M directly following the addition of DCMU, as described above. In both cases, low light intensities led to a sharp increase in the value of F_M towards that typical of State 1. This increase was observed for both red and far-red light illumination and appeared to reflect a simple reversal of dark adaptation. Higher intensities of red light tended to reverse this initial increase and decrease the value of F_M to a value typical of State 2, whilst higher intensities of far-red light led to a more complete State 1 adaptation (Fig. 5a). Differences in the fractions of red and far-red light absorbed by the algae make direct comparisons of intensity dependence difficult. The experiments were, therefore, repeated using red light in the presence of DCMU rather than far-red light to drive State 1 adaptation (Fig. 5b). Essentially similar results were obtained.

Two independent light-driven processes appear to be occurring in these experiments; a wavelength-independent process unaffected by DCMU in which the cells undergo a transition from dark to State 1 and, superimposed on top of this process, the normal DCMU-sensitive State 1/State 2 change. The former transition is triggered by extremely low levels of illumination (not over 200 mW · m^{-2}) equivalent to approx. 0.05% of saturation intensity for photosynthesis. The higher-light requirement for State 2 adaptation suggests that a certain level of illumination is required to set the redox level of the plastoquinone pool to an appropriately reduced state and to allow significant ATP synthesis. The extremely low intensity requirement of dark/State 1 adaptation suggests that this process is controlled in some quite different way.

Uncoupler and ATPase inhibitor studies

State 2 adaptation is believed to be associated with the phosphorylation of LHC II and is inhibited by the addition of uncouplers that block ATP production. Under these conditions, LHC II is rapidly dephosphorylated by the LHC-II-phosphatase system [8]. If the low fluorescence yield of PS II in dark-adapted algae were the result of a similar phosphorylation of LHC II, lowering the intracellular ATP levels by the addition of uncouplers or ATPase inhibitors would be expected to result in an increase in fluorescence yield to that typical of State 1.

The effects of adding the uncoupler CCCP or the ATPase inhibitors DCCD and tributyltin chloride to dark-adapted algae are shown in Figs. 6 and 7. If the additions were made together with the DCMU, they suppressed the normal slow light-dependent fluorescence increase seen in these cells. If, however, they were made after this increase was completed, the fluorescence yield dropped back to that typical of the dark state even in illuminated cells. It is thus quite clear that the low fluorescence yield of dark-adapted algae cannot be attributed to the phosphorylation of LHC II.

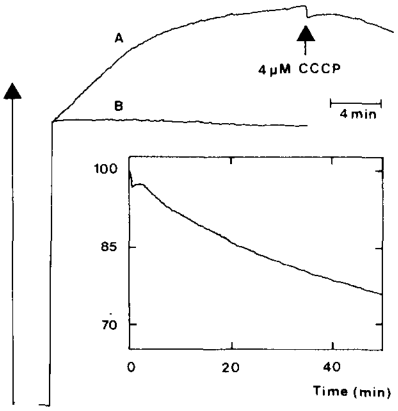

Fig. 6. Inhibition and reversal of light-driven fluorescence increases in poisoned algae. Dark adapted algae were poisoned with DCMU (6 μm) as in Fig. 2. 4 μM CCCP was either added together with DCMU (curve B) or after completion of the light-dependent rise (curve A). The decay of fluorescence in the light of samples of the latter type, expressed as percentage reduction of maximal fluorescence yield, is shown in the inset.

Addition of these reagents to algae preadapted to State I or State II prior to DCMU poisoning (not shown) resulted in essentially identical results. In all cases, the fluorescence yield decayed to that typical of dark-adapted algae. The rate of fluorescence decay in such samples is very slow with typical half-times of the order of 10–20 min. These rates are clearly not reflections of slow penetration of the uncoupler or inhibitors as they are immediately effective in suppressing the fluorescence increase if added together with DCMU and presumably reflect the rate of membrane reorganisations taking place as the algae relax to a metabolic state equivalent to the dark-adapted state.

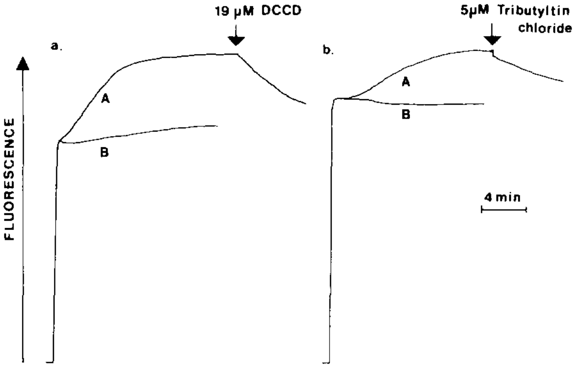

Fig. 7. Inhibition and reversal of light-driven fluorescence increases in poisoned algae. Measurements were performed as described in the caption to Fig. 6 using; (a) 19 μM DCCD added together with the DCMU (curve B) or after completion of the light-dependent rise (curve A); (b) analogous curves measured using 5 μM tributyltin chloride.

It is important to remember that uncouplers and ATPase inhibitors of the type used here are likely to have other non-specific effects on algal metabolism and that these may also lead to changes in fluorescence yield. The fact that these agents all led to similar changes in fluorescence yield with broadly similar kinetics suggests, however, that such indirect effects are unlikely to be of importance in the present experiments.

Light-dependent fluorescence increases of the type described here are also seen in blue-green and red algae (see references in Ref. 18). These changes show a similar sensitivity to uncouplers and Fork and Satoh [29] have suggested that they are related to proton fluxes associated with cyclic electron transport. Such changes have been reported to be insensitive to the addition of the ATPase phlorizin [28,29]. Whilst we also find no effect of phlorizin on *Chlorella* fluorescence, control measurements involving measurements of steady-state oxygen evolution suggest that this inhibitor penetrates algal cells to only a very limited extent. The results presented in Fig. 7 clearly indicate that the light-dependent fluorescence increases seen in *Chlorella* are inhibited by DCCD and tributyltin chloride. It should be emphasised, however, that it is still not certain whether this reflects an ATP requirement for such changes or secondary effects of these inhibitors on cyclic electron flow. The very low light-intensity requirement for these changes (Fig. 5) would tend to suggest that the latter is the more probable.

Discussion

In terms of the LHC-II phosphorylation model, preferential absorption of light in PS II leads to the activation of the LHC-II kinase, the phosphorylation of LHC II, the transfer of excess excitation energy from PS II to PS I and the establishment of State 2. Preferential absorption of light in PS I, in contrast, leads to the switching off of the kinase, the dephosphorylation of LHC II, a minimisation of excitation energy transfer from PS II to PS I and the establishment of State 1 [9,11]. The slow fluorescence increase seen following the addition of DCMU to State-2-adapted *Chlorella* is readily interpreted, on this basis, as a reflection of the dephosphorylation of LHC II as the algae relax to State 1. Problems arise, however, when this model is extended to cover the dark state. The relationship between State 1, State 2 and the dark-adapted state in algae has been the subject of considerable controversy. Bonaventura and Myers [1] initially reported that *Chlorella* was in State 1 in the dark. Subsequent studies in this and other laboratories have shown that dark adaptation leads to a state more closely related to State II [18,30,31]. A comparable pattern of behaviour is seen for blue-green [14,29,32] and red algae [33,34]. Dark-adapted blue-green and red algae show similar, but more rapid, changes in fluorescence yield following the additon of DCMU to those seen in green algae. These algae lack LHC II but it is usually assumed, although there is no direct evidence to support such an assumption, that they contain some component that plays a role analogous to LHC II.

Measurements of room temperature fluorescence yields associated with PS II (Fig. 2) and low temperature (77 K) emission spectra (Fig. 3) confirm that dark adaptation of *Chlorella* cells, like State-2-adaptation, leads to a redistribution of excitation energy in favour of PS I. If, however, LHC-II phosphorylation is to be invoked to explain this redistribution, two conditions would need to be met. The LHC-II kinase system would need to be active in the dark and a suitable source of ATP would be required for LHC II phosphorylation. The kinase system is normally inactive in the dark under in vitro conditions [11] reflecting the fact that the plastoquinone pool tends to become oxidised under such conditions. The redox state of the plastoquinone pool of dark-adapted algal cells is less clear. Whilst it becomes reduced under anaerobic conditions, its state under normal conditions has not yet been properly defined. The situation regarding the availability of ATP is also unclear although a recent report by Bennoun [35] of the existence of a 'chlororespiratory' pathway in algal chloroplasts suggest that this may not be a problem. Our observations that the addition of uncouplers and ATPase inhibitors lead to a suppression of the light-dependent fluorescence increase seen in DCMU-poisoned cells and a reduction in the fluorescence yield of such cells to a level typical of the dark state (Figs. 6 and 7), however, argues strongly against the idea that LHC

II is phosphorylated in the dark state.

Despite the fact that State 2 and the dark state show many similarities, these two states thus appear to be fundamentally different in origin. This view is strongly supported by the measurements of the intensity dependence of the State 1/State 2 adaptation of previously dark-adapted algae presented in Fig. 5, which indicate the existence of two light-dependent mechanisms for the control of excitation-energy distribution in photosynthesis, one involving a light/dark change and the other State 1/State 2 adaptation.

A scheme showing the possible relationship between the two mechanisms for controlling excitation energy distribution is presented in Fig. 8. In terms of this scheme, the dark/light changes reflect alterations in the ionic environment of the chloroplast triggered by electron-transport related proton movements which lead to ion-induced changes in thylakoid membrane organisation. The precise nature of these changes, it must be emphasised, is not known. Electron transport is, however, known to lead to an influx of protons into, and a counter-ion flux of Mg^{2+} out of, the intrathylakoid space of intact chloroplasts [36]. The distribution of excitation energy between the two photosystems is particularly sensitive to Mg^{2+} levels [12] and one obvious possibility is that dark-adaptation results in an accumulation of Mg^{2+} into the intrathylakoid space, and hence an increase in excitation-energy transfer between the two photosystems, whilst illumination leads to a release of Mg^{2+} and a corresponding reduction in such transfer.

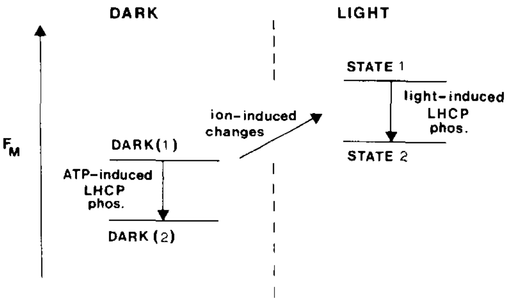

Fig. 8. Diagrammatic model illustrating the relationship between dark/light transition associated with divalent cation movements and State 1/State 2 transitions associated with LHC-II phosphorylation. See text for details.

Under in vivo conditions, State 1/State 2 changes associated with LHC-II phosphorylation are necessarily superimposed on top of the ion-induced changes as light is required both to set the plastoquinone pool to an appropriate redox level and to produce ATP. Under in vitro conditions, however, the two processes can be divorced by use of exogenous ATP and State-2 adaptation can take place even in the dark if appropriate reducing agents are used to activate the kinase [11]. This would then correspond to a transition between the states designated dark (1) and dark (2) in Fig. 8.

Horton and Black [37] have recently demonstrated the synergistic effects of ATP and Mg^{2+}-level changes on the fluorescence yield of isolated pea chloroplasts. They compared the effect of ATP addition under low Mg^{2+} (1 mM) and high Mg^{2+} (5 mM) conditions. Lowering the Mg^{2+} concentration resulted in a value of F_M for non-phosphorylated chloroplasts slightly lower than that for the phosphorylated chloroplasts in the presence of the higher Mg^{2+} concentration. This, in our opinion, is directly analogous to the situation in *Chlorella* where the non-phosphorylated dark-adapted algae show a lower fluorescence yield than the phosphorylated State 2-adapted algae.

The light-dependent fluorescence increases seen on the addition of DCMU to dark and State-2-adapted cells are, on this basis, fundamentally different in origin; the increase seen for the dark-adapted cells reflecting changes in the ionic environment of the photosynthetic membranes and that for State-2-adapted cells a dephosphorylation of LHC II. Their similarity merely reflects the fact that they involve rather similar changes in thylakoid organisation. This explains why exposure to light preferentially absorbed in PS II apparently has little or no effect on dark-adapted algae: the fluorescence yield decrease associated with LHC-II phosphorylation being largely compensated by the ion-induced fluorescence increase. It may also explain reports that *Scenedesmus* mutants lacking LHC II still appear to undergo State 1/State 2 changes [38]. Absence of LHC II clearly does not necessarily preclude dark/light changes as such changes are seen in blue-green algae [39]. It is, therefore, possible that it is these changes, rather than State 1/State 2 changes that are observed in the mutants.

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