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COMPARISON OF ATP-INDUCED AND STATE 1/STATE 2-RELATED CHANGES IN EXCITATION ENERGY DISTRIBUTION IN *CHLORELLA VULGARIS*

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The addition of ATP to thylakoids isolated from *Chlorella vulgaris* is shown to lead to a quenching of fluorescence originating from Photosystem II and phosphorylation of chlorophyll *a*/chlorophyll *b* light-harvesting protein (LHCP) directly analogous to that reported for higher-plant chloroplasts. The time courses of these two processes are shown to be identical. Parallel measurements of ATP-induced changes in the fluorescence properties of isolated algal thylakoids and light-driven (State 1/State 2 changes) in whole cells strongly support the idea that LHCP phosphorylation plays an important role in State 2 adaptation under in vivo conditions.

Introduction

The concept of State 1/State 2 changes was first introduced by Bonaventura and Myers [1]. They observed 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 photoprocesses associated with PS II whilst cells illuminated with light preferentially absorbed in PS II undergo a transition to a state (State 2) characterised by a high efficiency of photoprocesses associated with PS I. The effect of these transitions is, in both cases, to maximise the photosynthetic efficiency of the algae under the prevailing light conditions. Recent studies [2–5] have demonstrated that the addition of ATP to chloroplasts isolated from higher plants leads to changes in Chl *a* fluorescence similar to those accompanying State 2 adaptation in whole-cell systems. These ATP-induced changes are associated with the reversible phosphorylation of Chl *a*/Chl *b* light-harvesting protein (LHCP) and appear to be regulated by the redox state of the plastoquinone pool lying between the two photosystems. Present evidence suggests that the transition from State 1 to State 2 is linked to a phosphorylation of LHCP under conditions in which PS I activity becomes rate limiting and the plastoquinone pool is reduced [4,5].

State 1/State 2-related changes in oxygen evolution and photosynthetic enhancement [1,6,7], Chl *a* fluorescence yield [1,8–14], fluorescence induction [9,10,12] and low-temperature (77 K) fluorescence emission [8,10,13,15] have been extensively studied in algal systems. Reports of State 1/State 2-related changes in the fluorescence yield of intact leaf tissue have also appeared recently [16,17], but associated changes in other variables are much more difficult to study in leaves. The study of ATP-induced changes in excitation en-

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Abbreviations: PS, photosystem; Chl, chlorophyll; LHCP, chlorophyll *a*/*b* light harvesting protein; Tricine, *N*-tris(hydroxymethyl)methylglycine, DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol.

ergy distribution, in contrast, has been restricted to measurements performed on chloroplasts isolated from higher plants. No comparable measurements on algal systems have, as yet, appeared. In this paper we provide a direct comparison between light-induced changes in room-temperature fluorescence yield, fluorescence induction and low-temperature (77 K) fluorescence emission spectra of *Chlorella vulgaris* cells and the corresponding ATP-induced changes in thylakoid preparations isolated from such cells.

Materials and Methods

Alga culture and thylakoid isolation. C. vulgaris was cultured as described previously [12]. Thylakoids were prepared from freshly grown cells as follows. The cells were first washed in 1 mM EDTA, 10 mM Tris-HCl (pH 7.2), 0.25 M sorbitol and then resuspended in an assay medium containing 25 mM Tricine (pH 7.8), 0.25 M sorbitol. They were then ruptured in a French pressure cell (800 $kg \cdot cm^{-2}$). Unbroken cells were removed by centrifugation at $750 \times g$ for 5 min. The supernatant was recentrifuged at $6500 \times g$ for 2.5 min and the pellet containing the isolated thylakoids was resuspended and washed in assay medium containing 5 mM $MgCl_2$. Thylakoids were normally stored in concentrated form in this medium during the course of experiments and diluted directly prior to use.

Phosphorylation measurements. Washed thylakoids were labelled using [γ-^{32}P]ATP as follows. Thylakoids (50 μg Chl) were resuspended in 0.5 ml of 0.25 M sorbitol, 5 mM NH_4Cl, 5 mM $MgCl_2$, 25 mM Tricine-KOH (pH 7.8). [γ-^{32}P]ATP was added to give a final ATP concentration of 500 μM at a specific activity of 100 $\mu Ci/\mu mol$. Protein phosphorylation was conducted for 5 min at 20°C in strong red light ($\lambda = 650$ nm; intensity approx. 6 $W \cdot m^{-2}$). After the incubation, the thylakoids were diluted with 10 ml of 50 mM Tris-HCl (pH 8.0), 1 mM Na_2EDTA, 50 μM phenylmethylsulphonyl fluoride (PMSF) and then centrifuged at $20000 \times g$ for 10 min. The pellet was washed in the same buffer and resuspended in 2.6 mM Tris, 48 mM glycine, 1% SDS at an SDS/Chl ratio of 10 : 1 [18]. After centrifugation for 10 min, the solution was incubated for 10 min at 20 or 70°C and then analysed for phosphorylated chlorophyll-protein complexes by electrophoresis of 10-μl samples through a 5% polyacrylamide gel containing SDS-Tris-glycine as described elsewhere [18]. After electrophoresis, the unstained gel was fixed, dried and autoradiographed [19].

Parallel measurements of chlorophyll-protein phosphorylation and fluorescence changes induced by the addition of [γ-^{32}P]ATP were performed as follows. Thylakoids (20 μg Chl) were suspended in 2 ml of assay medium (25 mM Tricine-KOH (pH 7.8), 0.25 M sorbitol, 5 mM NH_4Cl, 10 mM $MgCl_2$) in the sample cuvette of an Applied Photophysics filter fluorimeter. [γ-^{32}P]ATP was added to the sample to yield a final concentration of 200 μM and 30 $\mu Ci/\mu mol$. The fluorescence yield of the sample was continuously monitored. 100-μl samples of the reaction medium were removed at fixed intervals and mixed with trichloracetic acid. The samples were subsequently analysed for phosphorylated chlorophyll-protein as described elsewhere [20].

Fluorescence measurements. All fluorescence measurements, apart from those described above, were made using a spectrofluorimeter constructed in this laboratory [13]. This instrument was normally used in an a.c. mode. In this mode a 62 Hz measurement beam is used to monitor fluorescence. The instrument is fitted with two much stronger d.c. light sources which can be used to pre-adapt the algae to a given state or to drive State 1/State 2-related fluorescence changes as appropriate. Room-temperature fluorescence measurements were made on samples (2–4 μg Chl/ml) held in 10-mm fluorimeter cuvettes. Low-temperature (77 K) measurements were made on samples (approx. 15 μg Chl/ml) sandwiched between two cover slips with a 0.5 mm Teflon spacer. Phycocyanin, isolated from *Anacystis nidulans* by a slightly modified form of the procedure described by Gershoni and Ohad [21], was used as an internal standard to allow normalisation of the low-temperature spectra. Fluorescence induction curves were recorded using the instrument in a d.c. mode using one of the strong d.c. light sources to pre-adapt the samples and the other as exciting source. The induction curves were recorded using a Tektronix 5115 oscilloscope fitted with a Paloroid camera.

Oxygen evolution. Whole algae and isolated thylakoids were assayed for electron-transport activity using a Hansatech D.W. oxygen electrode unit.

Electron microscopy. Specimens fixed in 5% glutaraldehyde and 2% osmium tetroxide in cacodylate buffer, pH 7.2, were sectioned, stained with lead citrate and uranyl acetate and then viewed in a Philips 301G electron microscope.

Results

Thylakoid characterisation

Thylakoids were isolated from *Chlorella* according to the procedure described in Materials and Methods. An electron micrograph showing a typical view of such thylakoids is presented in Fig. 1a. The algal chloroplasts are clearly extensively disrupted by the isolation procedure. The samples appear to consist mainly of partially stacked broken chloroplasts. Little contamination from unbroken cells or small thylakoid fragments was observed but the samples did contain appreciable amounts of cell-wall debris. There is, however, no reason to believe that such debris is of importance for the results we describe. A higher magnification view of a stacked region of the isolated membranes is shown in Fig. 1b.

Typical values for the electron-transport activities of the preparations are set out in Table I. These rates, which are about 20–40% of the electron-transport rate of the intact algae, are very similar to those reported in earlier studies on isolated algal chloroplasts [22]. Two parameters of especial interest in this study are the Mg^{2+}-induced increase in fluorescence yield accompanying thylakoid stacking and the ATP-induced fluorescence decrease associated with LHCP phosphorylation. The extents of these changes in the thylakoid fraction used in this investigation and the low-spin and supernatant fractions of the pressed cells discarded during thylakoid isolation are compared in Table II. The largest changes are observed for the thylakoid fraction. The changes observed for the low-spin fraction (consisting mainly of unbroken cells together with some isolated thylakoids) and the supernatant fraction (consisting mainly of very small thylakoid fragments) were by comparison negligible.

TABLE I

ELECTRON-TRANSPORT RATES OF *CHLORELLA* THYLAKOID PREPARATIONS

Reaction	Electron-transport rate [a] (equiv./mg Chl per h)
$H_2O \rightarrow$ ferricyanide	204
$H_2O \rightarrow$ methyl viologen	106
$DCIPH_2 \rightarrow$ methyl viologen	128

[a] Average of six estimations in each case.

LHCP phosphorylation

In order to check whether LHCP is phosphorylated in vitro in *Chlorella*, isolated thylakoids were incubated with [γ-^{32}P]ATP and then subjected to polyacrylamide gel electrophoresis in SDS-Tris-glycine. This procedure when applied to tobacco and pea thylakoids yields four major chlorophyll-protein complexes together with very little free chlorophyll [18,19]. The slowest migrating band termed A-1 contains only Chl *a* and the three more rapidly migrating bands AB-1, AB-2 and AB-3 contain both Chl *a* and Chl *b*. When applied to *Chlorella* thylakoids, the procedure yields two major chlorophyll-protein complexes (A, equivalent to A-1, and AB, equivalent to AB-3) and again very little free chlorophyll (Fig. 2, panel b). The two complexes are stable at 20°C but at 70°C they dissociate to generate free chlorophyll (Fig. 2,

TABLE II

Mg^{2+}-INDUCED AND ATP-INDUCED FLUORESCENCE YIELD CHANGES FOR DIFFERENT CELL FRACTIONS ISOLATED DURING THYLAKOID PREPARATION

Cell fraction	Fluorescence yield change (%)	
	Mg^{2+}-induced [a]	ATP-induced [b]
Cell debris and unbroken cells (750 × *g* pellet)	+14.0	−0.4
Thylakoid fraction (6500 × *g* pellet)	+76.5	−20.9
Thylakoid fragments (6500 × *g* supernatant)	−12.2	+2.2

[a] Changes in F_m measured on adding Mg^{2+} (final concentration 10 mM) to thylakoids in Mg^{2+}-free assay medium containing 6 μM DCMU.

[b] Changes measured on adding ATP (final concentration 0.5 mM) to thylakoids in assay medium containing 10 mM Mg^{2+}.

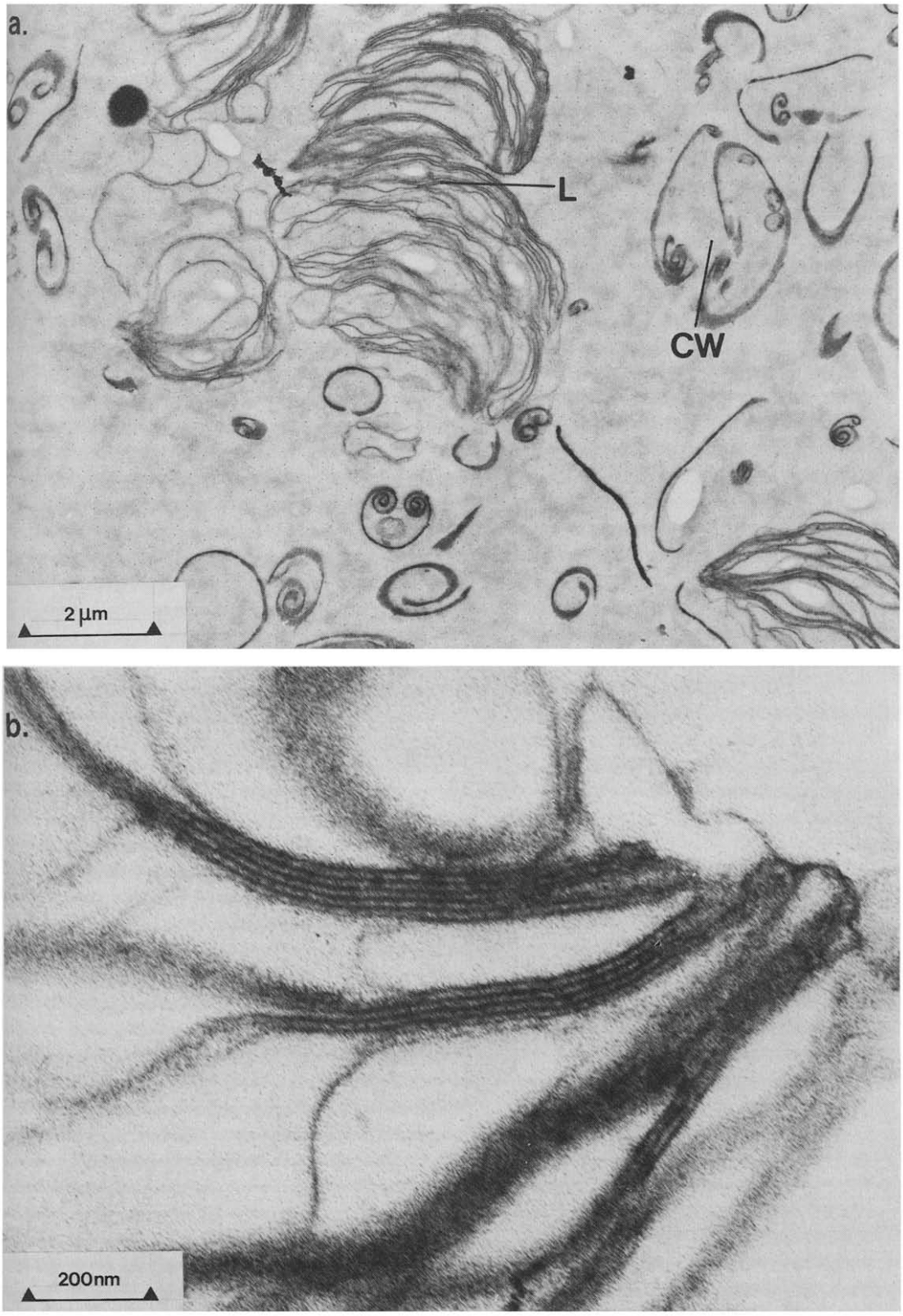

Fig. 1. Electron micrographs showing (a) The general composition of the *Chlorella* thylakoid preparations used in fluorescence studies. Samples consisted largely of large lamellar fragments (L) and cell-wall debris (CW). (b) A higher magnification view of a stacked region of *Chlorella* lamellae. Thylakoids were suspended in assay medium (25 mM Tricine (pH 7.8), 0.25 M sorbitol) containing 10 mM $MgCl_2$.

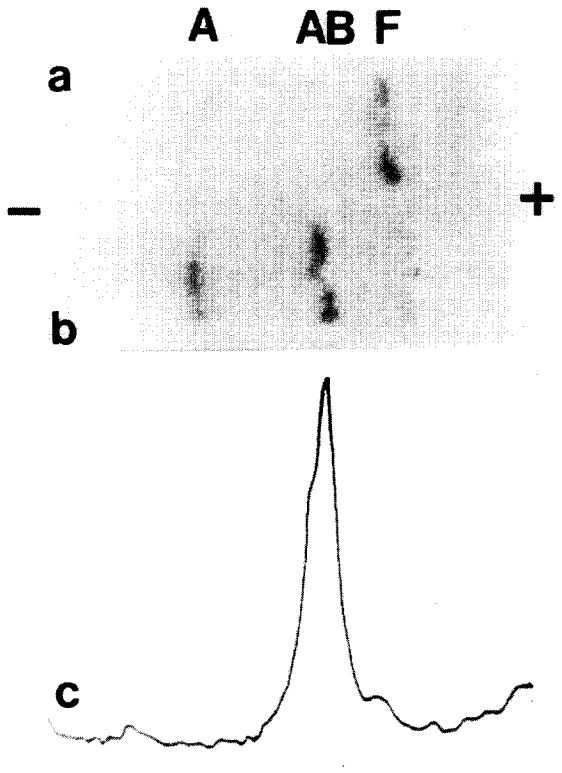

Fig. 2. Phosphorylation of Chl *a*/*b*-protein complex of *Chlorella*. Isolated thylakoids were incubated with [γ-^{32}P]ATP as described in Materials and Methods, solubilized with SDS at 70°C (panel a) or 20°C (panels b and c), and analysed by polyacrylamide gel electrophoresis in SDS-Tris-glycine at 20°C. The gel was fixed without staining, dried and autoradiographed. Panels a and b, photograph of gel showing migration of two major chlorophyll-protein complexes (A and AB) and free chlorophyll (F). Panel c, scan of autoradiogram corresponding to panel b.

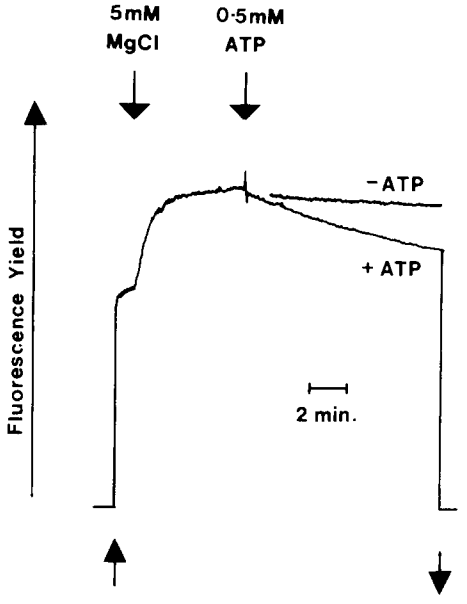

Fig. 3. Trace showing the effects of added Mg^{2+} and ATP on the fluorescence yield of *Chlorella* thylakoids suspended in assay medium (25 mM Tricine (pH 7.8), 0.25 M sorbitol) containing 5 mM NH_4Cl as uncoupler. Excitation wavelength 440 nm; detection wavelength 685 nm.

panel a). Autoradiography of the gel shows that the major phosphoproteins co-migrate, as expected, with complex AB (Fig. 2, panel c).

Fluorescence measurements

Addition of ATP to thylakoids isolated from *Chlorella* leads to changes in Chl *a* fluorescence very similar to those observed for higher-plant chloroplasts. A typical trace illustrating the ATP-induced changes for *Chlorella* is shown in Fig. 3. The thylakoids, which were uncoupled with NH_4Cl (5 mM), were initially suspended in Mg^{2+}-free medium. Addition of $MgCl_2$ (5 mM) leads to a rapid increase in fluorescence yield of the type normally seen for broken higher-plant chloroplasts. On adding ATP (0.5 mM), the fluorescence yield of the sample slowly decreases. The half-time ($t_{1/2} \simeq 3$–4 min) and the extent (15–20%) of this decrease are both very similar to those reported for higher-plant chloroplasts [2–5]. If DCMU (6 μM) is added after equilibrium with ATP (not shown) only small (5–10%) increases in fluorescence yield are observed. This indicates that the measurements are made at fluorescence levels close to the maximal yield, F_m, and that the ATP-induced fluorescence decrease cannot be attributed to changes in the variable components of fluorescence, F_v, associated with changes in the fraction of open PS II traps. Addition of DCMU prior to the addition of ATP completely blocks the ATP-induced changes.

The results of parallel measurements of ATP-induced fluorescence changes and phosphorylation of LHCP are set out in Fig. 4. The time dependencies of the two phenomena are very similar. Any discrepancy between the two time courses can reasonably be attributed to thylakoid deterioration

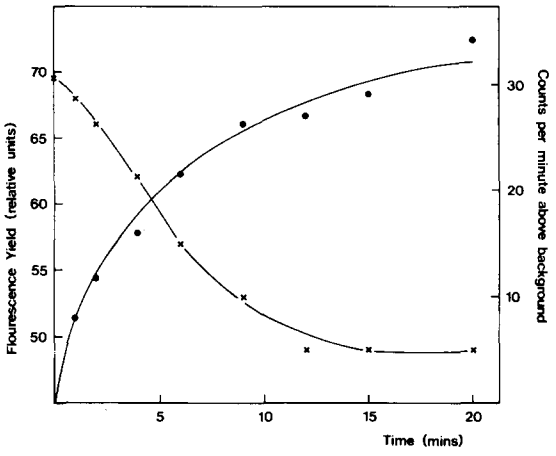

Fig. 4. Plot showing simultaneous measurement of the time courses of ATP-induced fluorescence quenching (×) and LHCP phosphorylation (●) in *Chlorella* thylakoids. See Materials and Methods for experimental details.

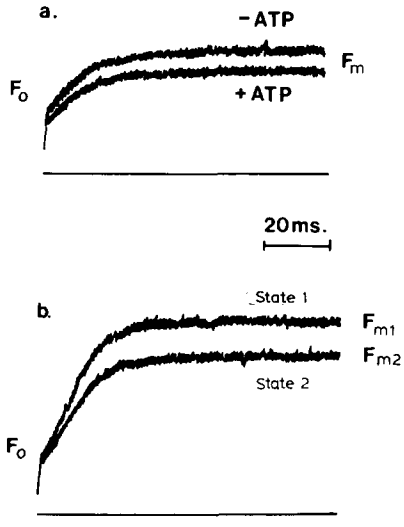

Fig. 5. Traces showing fast fluorescence induction curves of (a) *Chlorella* thylakoids suspended in assay medium containing 10 mM $MgCl_2$ and 5 mM NH_4Cl following incubation for 10 min in red light (650 nm) in the presence, or absence, of 0.5 mM ATP. (b) Whole cells pre-adapted to State 2 by exposure to red light ($\lambda = 650$ nm: intensity 3.8 $W \cdot m^{-2}$) or to State 1 by exposure to far-red light ($\lambda = 702$ nm; intensity 2.9 $W \cdot m^{-2}$) for 20 min. In each case the pre-adapted samples were kept in the dark for 1 min, 6 μM DCMU added and sample allowed to equilibrate for a further 30 s before measurement.

during the time course of the measurement. Such deterioration tends to lead to a slow ATP-independent reduction in chlorophyll fluorescence as illustrated in the control curve shown in Fig. 3.

The initial Mg^{2+}-induced increase in fluorescence yield seen in Fig. 3 reflects divalent-ion-induced changes of the type commonly observed in higher-plant chloroplast preparations [23]. Similar changes are observed if Ca^{2+} is used in place of Mg^{2+} or if higher concentrations of monovalent cations such as Na^+ or K^+ are employed. The much greater effectiveness of divalent as opposed to monovalent cations, as pointed out by Barber [24], is characteristic of charge shielding effects. The extent of ATP-induced fluorescence decreases of the type shown in Fig. 3 varied somewhat from preparation to preparation. It was noted, however, that thylakoid samples that showed large Mg^{2+}-induced fluorescence increases almost invariably showed large ATP-induced decreases whilst samples showing small Mg^{2+}-induced changes showed little or no ATP-induced changes. As the samples aged, their ability to show Mg^{2+}- and ATP-induced fluorescence changes both decreased.

Fluorescence induction curves for whole cells first adapted to State 1 or State 2 and then poisoned by the addition of DCMU are shown in Fig. 5b. Corresponding curves for isolated thylakoids, measured in the presence and absence of ATP, are shown in Fig. 5a. The curves obtained for isolated thylakoids are noticeably less sigmoidal than those for whole cells. The ratio of F_m/F_0 for these samples is also much lower. Nevertheless, the general effects of State 2 adaptation in whole cells and ATP addition in isolated thylakoids appear to be very similar. In both cases, reductions in fluorescence yield occur at the F_m and F_0 levels. These observations are in agreement with earlier findings of Horton and Black [25], working with pea chloroplasts, but contrast strongly with the recent findings of Kyle et al. [26]. The latter authors report that whilst the value of F_m decreases on addition of ATP that of F_0 shows a small increase.

We have not attempted a full analysis of our fluorescence induction curves into α- and β-components of the type proposed by Melis and Homan [27]. In our experience such analyses are extremely sensitive to the choice of the limiting fluorescence level. Accurate determination of this value is particularly difficult in the case of *Chlorella* as dark-adapted, DCMU-poisoned cells undergo slow light-driven changes in F_m associated

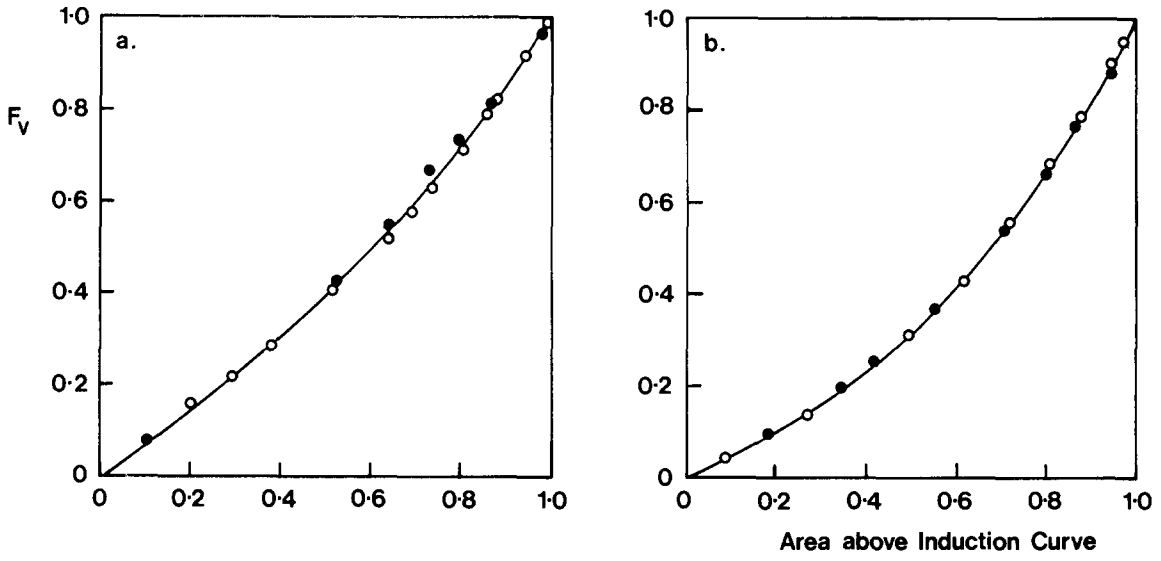

Fig. 6. Plots of the normalised area above the induction curves shown in Fig. 6 as a function of the normalised value of F_v. (a) Isolated thylakoids measured in the presence of (●) and absence (○) of 0.5 mM ATP (b) Whole cells pre-adapted to State 2 (●) and State 1 (○).

with State 1/State 2 transitions [12]. Preliminary analyses of our data, using the value of F_m measured after 80 ms as the limiting value, yielded curves very similar to those reported by Horton and Black [25]. Little or no change in the shape of plots of F_v against the area above the induction curve was detected in response to ATP addition to thylakoids or State 2 adaptation in algae (Fig. 6). We cannot, however, exclude the possibility that the fluorescence changes associated with β-centres are not completed within the time scale of our measurements and that more detailed analysis might reveal changes in the ratio of α- to β-centres of the type reported by Kyle et al. [26].

It is well established that ATP addition to chloroplasts [4] and State 2 adaptation of whole cells [7] lead to an increase in the ratio of fluorescence emission from PS I to that from PS II at low temperatures (77 K). Owing to difficulties in the normalisation of such spectra, it has proved difficult to demonstrate that these procedures lead to a simultaneous decrease in PS II and increase in PS I emission. This problem has, however, recently been resolved by Krause (personal communication) by the simple expedient of including fluorescein in his samples as an internal fluorescence standard. His results show conclusively that ATP addition to intact pea chloroplasts leads to an increase in PS I and a decrease in PS II.

A comparison of the effects of ATP addition and State 2 adaptation on the low-temperature emission spectra of thylakoids and whole cells of *Chlorella* is presented in Fig. 7. We were unable to use fluorescein in the present study as its use necessitates the addition of glycerol to prevent ice-crystal formation and glycerol addition, probably as a result of osmotic effects, inhibits State 1 adaptation in *Chlorella* cells. Following the procedure of Gershoni and Ohad [21], phycocyanin was used as an alternative standard and the spectra were normalized to its emission peak. The spectra obtained for thylakoids and whole cells showed some slight differences. The 685 nm emission peak (associated with PS II) was normally lower with respect to the 720 emission peak (associated with PS I) in the thylakoid samples. This, as noted above, probably reflects stacking differences in the two types of preparation. It is quite clear, however, that ATP-addition to thylakoids and State 2 adaptation of whole cells lead to reductions in PS II emission and increases in PS I emission consistent with a redistribution of excitation energy in favour of PS I.

Allen et al. [4] and Horton et al. [5] have

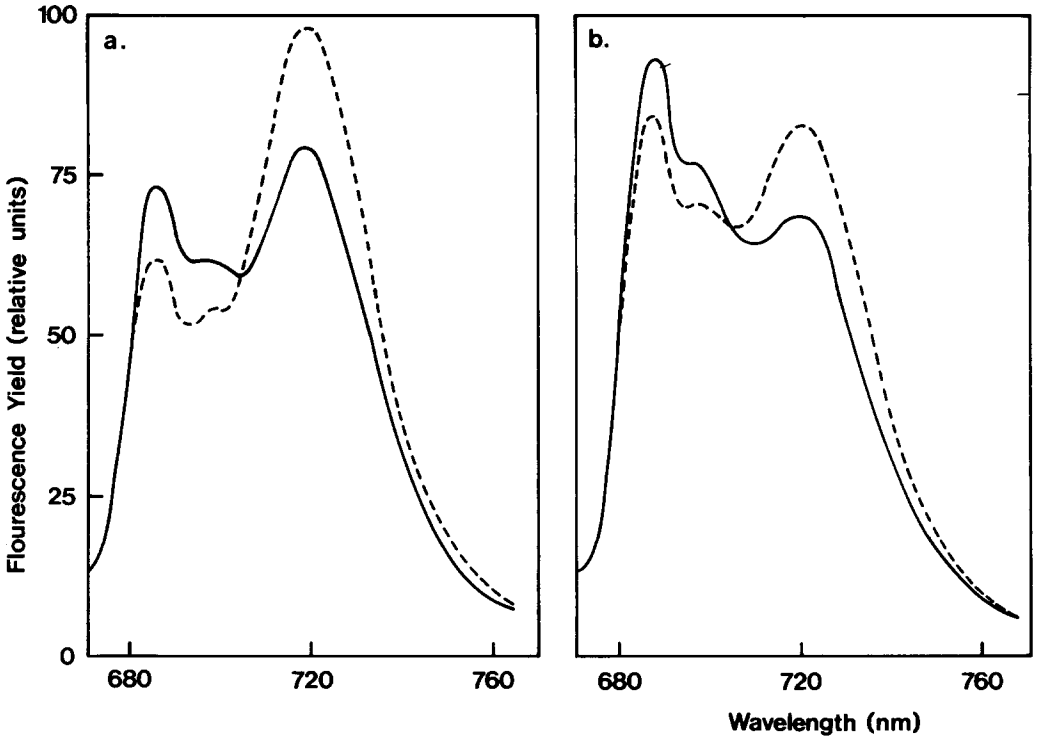

Fig. 7. Low-temperature (77 K) emission spectra of (a) *Chlorella* thylakoids suspended in assay medium containing 10 mM $MgCl_2$ and 5 mM NH_4Cl. Samples were incubated in red light (650 nm) in the presence, or absence, of 0.5 mM ATP prior to freezing. (b) *Chlorella* whole cells pre-adapted to State 2 or State 1 as described in the legend to Fig. 5, prior to freezing. Phycocyanin was added to all samples as an internal standard and emission spectra were normalised to its low-temperature emission peak. Spectra are not corrected for photomultiplier response. Excitation wavelength 560 nm. The full lines correspond to the spectra of thylakoids measured in the presence of ATP or whole cells pre-adapted to State 2 and the dashed lines to those of thylakoids measured in the absence of ATP or whole cells adapted to State 1.

TABLE III

SUMMARY OF FLUORESCENCE INDUCTION AND LOW-TEMPERATURE EMISSION DATA [a] FOR *CHLORELLA* THYLAKOIDS [b] AND WHOLE CELLS

System	F_m [c]	F_0 [c]	F_{720}/F_{685}
Whole cells			
State 1-adapted	100	29	1.15
State 2-adapted	82 (18%)	27.5 (5%)	1.48
Thylakoids			
Red preillumination (− ATP)	100	50	1.45
Red preillumination (+ ATP)	82.5 (17.5%)	46.5 (7%)	1.81
Far-red preillumination (− ATP)	100	45.5	1.51
Far-red preillumination (+ ATP)	98 (2%)	44.5 (2%)	1.48

[a] Excitation wavelength 440 nm.
[b] Samples contained 1 μM plastocyanin, 3.4 μM ferredoxin; 1 mM NADP, 1 $\mu g \cdot ml^{-1}$ NADP reductase.
[c] Normalised values; figures in brackets are percentage decreases.

recently demonstrated that the addition of ATP to pea chloroplasts leads to phosphorylation of LHCP, and hence to a lowering of fluorescence yield of Chl *a* associated with PS II, only if the plastoquinone pool is in a reduced state. In order to check whether the same situation pertains in *Chlorella*, we compared the effect of pre-illuminating our thylakoids with red and far-red light prior to the addition of ATP. The results of fluorescence induction and low-temperature emission measurements made under such conditions are summarised in Table III. In both cases the effect of far-red light, which tends to oxidise plastoquinone, was to inhibit the ATP effect. These measurements, it must be stressed, were performed in the presence of added plastocyanin, ferredoxin-NADP reductase and NADP to permit electron flow through PS I. In the absence of these additions, the effects of red and far-red light were normally identical, presumably reflecting the inability of far-red light to maintain the plastoquinone pool in an oxidised state in our thylakoid preparations.

Discussion

The ATP-induced changes in the fluorescence yield of thylakoids isolated from *Chlorella* reported in this paper are very similar to those previously reported for higher plant chloroplasts by other workers [2–5]. Addition of ATP to *Chlorella* thylakoids leads to changes in the steady-state fluorescence of Chl *a* associated with PS II (Fig. 3), in fast fluorescence induction (Figs. 5 and 6) and in low-temperature (77 K) emission (Fig. 7). All these changes are very similar to the corresponding changes occurring on State 2 adaptation of whole cells. These observations, together with the fact that the ATP-induced fluorescence changes observed for *Chlorella* thylakoids show a very similar time dependency to the phosphorylation of LHCP in such preparations (Fig. 4), strongly support the idea that LHCP phosphorylation plays a major role in State 2 adaptation in vivo. Similarly, our observation that the ATP-induced changes in fluorescence induction and low-temperature emission are inhibited by far-red light (Table III) adds further support to the suggestion of Allen et al. [4] and Horton et al. [5] that the kinase controlling such phosphorylation is only active when the plastoquinone pool is in a reduced state.

It is quite clear from enhancement studies [1,6] that the lowering of PS II photochemical activity associated with State 1 adaptation in whole cells is accompanied by a simultaneous increase in PS I activity. These changes reflect a redistribution of excitation energy between the two photosystems [1,7]. The question of whether or not ATP-induced fluorescence quenching of isolated chloroplasts is accompanied by increases in the efficiency of PS II photoprocesses has, however, been a question of some debate [28–30]. The normalised, low-temperature emission spectra reported by Krause (personal communication) for ATP-treated intact chloroplasts, together with the spectra reported here for algal cells and algal chloroplasts (Fig. 7), would seem to provide convincing evidence that LHCP phosphorylation does in fact lead to the anticipated redistribution of excitation energy between PS II and PS I.

One of the major outstanding questions regarding the mechanism of State 1/State 2-related changes in the distribution of excitation energy between the two photosystems is whether these changes involve a transfer of pigments from one photosystem to the other or a change in the efficiency of resonance energy transfer between PS II and PS I. The two mechanisms can be distinguished by the fact that energy transfer between neighbouring PS II light-harvesting units should be sensitive to changes in energy transfer between the photosystems but independent of pigment exchange [31]. The shape of the fluorescence induction curve of DCMU-poisoned algae is believed to reflect the efficiency of energy transfer between PS II units [23]. Measurements of the effect of State 2 adaptation and/or ATP addition on the shape of this curve should, in principle at least, resolve this point. Unfortunately, the results of such analyses are not identical. Horton and Black [25] observed little or no changes in the sigmoidicity of the induction curve on addition of ATP to pea chloroplasts whilst Kyle et al. [26] report marked changes. Our results (Fig. 6) tend to support those of Horton and Black but, as we have already stressed, this type of measurement is particularly difficult to perform for *Chlorella*.

Analysis of the shape of low-temperature fluorescence induction curves by Haworth et al. [33]

suggests that changes in the initial distribution of excitation energy between the two photosystems and in resonance energy transfer between the photosystems are both affected by LHCP phosphorylation. The possibility that the relative contribution of these two factors may differ under different assay conditions cannot, therefore, be excluded. A final resolution of the relationship between State 2 adaptation and LHCP phosphorylation is probably best deferred until a clearer picture emerges regarding the significance of these observations.

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