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STATE 1–STATE 2 TRANSITION IN LEAVES AND ITS ASSOCIATION WITH ATP-INDUCED CHLOROPHYLL FLUORESCENCE QUENCHING

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By using chlorophyll fluorescence, a study has been made of changes in spillover of excitation energy from Photosystem (PS) II to PS I associated with the State 1–State 2 transition in intact pea and barley leaves and in isolated envelope-free chloroplasts treated with ATP. (1) In pea leaves, illumination with light preferentially absorbed by PS II (Light 2) led to a condition of maximum spillover (state 2) while light preferentially absorbed by PS I induced minimum spillover condition (State 1) as judged from the redox state of Q and low-temperature emission spectra. The State 1–State 2 transitions took several minutes to occur, with the time increasing when the temperature was lowered from 19 to 6°C. (2) In contrast to the wild type, leaves of a chlorophyll *b*-less mutant barley did not exhibit a State 1–State 2 transition, suggesting the involvement of the light-harvesting chlorophyll *a*/*b*-protein complex in spillover changes in higher plants. (3) Spillover in isolated pea chloroplasts was increased by treatment with ATP either (a) in Light 2 in the absence of an electron acceptor or (b) in the dark in the presence of NADPH and ferredoxin. These observations can be interpreted in terms of the model that a more reduced state of plastoquinone activates the protein kinase which catalyzes phosphorylation of the light-harvesting chlorophyll *a*/*b*-protein complex (Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981). Nature 291, 25–29). This process was found to be very temperature sensitive. (4) Pea chloroplasts illuminated in the presence of ATP seemed to exhibit a slight decrease in the degree of thylakoid stacking, and an increased intermixing of the two photosystems. (5) The possible mechanism by which protein phosphorylation regulates the State 1–State 2 changes in intact leaves is presented in terms of changes in the spatial relationship of two photosystems resulting from alteration in membrane organization.

Introduction

The maximum quantum yield of steady-state O_2 evolution from photosynthetic organisms approaches a value expected for the operation of two photosystems [1]. These are known as PS I and PS II and they form the basic feature of the Z-scheme [2,3]. However, PS I and PS II do not have identical absorption spectra [4] so they can be unequally excited. In this event, it is advantageous for the redistribution of excitation energy to occur between the two photosystems so as to attain the optimum photosynthetic

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; LHCP, light-harvesting chlorophyll *a*/*b*-protein complex; PS, photosystem; Q, primary acceptor of PS II.

electron-transfer rate for a particular lighting condition. Such an adjustment has been reported to occur in intact algae [4–13] and involves changes in spillover of energy from the light-harvesting pigments of PS II to those of PS I [14,15]. When algae are exposed to light which is preferentially absorbed by PS II (Light 2) the organism slowly adjusts to the imbalance by allowing some of the excitation to be directed, by energy transfer, to PS I. This spillover state is known as State 2. On the other hand, when the illumination predominantly excites PS I (Light 1), spillover is maintained at a minimum and the alga is said to be in State 1. Thus, changes between State 1 and State 2 function to maximize the quantal efficiency of photosynthetic electron transport under limiting intensities for different spectral qualities of the incident light.

A possible biochemical mechanism for such a 'tuning' of the photosynthetic apparatus to the quality of incident light has emerged recently, largely as a result of work by Bennett and co-workers [16, 17] and Horton and Black [18,19] using isolated chloroplasts. The phosphorylation of LHCP in thylakoids catalyzed by a protein kinase, and the dephosphorylation catalyzed by a phosphatase have been studied in some detail [20–26]. Phosphorylation of LHCP was found to increase the spillover of excitation energy from PS II to PS I, whereas dephosphorylation had the reverse effect [16–20]. Further, it was found that the redox state of plastoquinone controlled the activity of the protein kinase by some as yet unknown mechanism [17,18]. Thus, it seems quite possible that the redox state of plastoquinone, which is an electron carrier between PS II and PS I, serves as a detector of the imbalance of excitation of the two photosystems, while the kinase/phosphatase activities provide the rectification of the imbalance. Such a hypothesis has emerged from experiments on isolated chloroplasts. The present study reports the existence of State 1–State 2 changes in intact leaves of higher plants as monitored by a modulated fluorescence technique [27] and compares these observations with the ATP-induced decrease in chlorophyll fluorescence associated with the phosphorylation of LHCP [20].

Materials and Methods

Measurements of relative chlorophyll fluorescence yield in leaves were made by exciting fluorescence with modulated blue-green light (Light 2) of irradiance 1.3 W/m^2 passed by Schott BG18 (4 mm) + BG38 (2 mm) filters. The modulated fluorescence at 686 ± 11 nm was detected by a photomultiplier and lock-in amplifier, as shown in Fig. 1. When required, non-modulated Light 1 at 710 ± 12 nm was introduced at an irradiance of about 7.5 W/m^2. A leaf attached to the plant was placed on an aluminium block, the temperature of which could be lowered if needed. A glass slide gently resting on the leaf helped to maintain a more uniform temperature around the leaf.

To obtain chlorophyll fluorescence spectra at 77 K, a disc was cut from the illuminated region of a leaf at the end of a light treatment, rolled into a cylinder, illuminated with Light 2 (20 W/m^2) for 15 s to reduce Q, and then frozen in liquid nitrogen. Samples in cylindrical quartz tubes bathed in liquid nitrogen

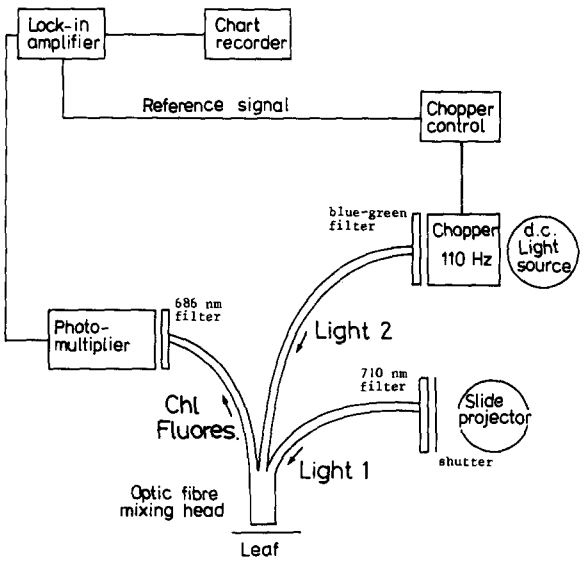

Fig. 1. Experimental arrangement for measuring State 1–State 2 transitions in leaves. 'Light 2', of irradiance 1.3 W/m^2, was transmitted by blue-green Schott filters BG18 plus BG38 and modulated at 110 Hz. 'Light 1', of irradiance 7.5 W/m^2, was transmitted by a 710 nm interference filter. The modulated chlorophyll fluorescence signal, transmitted by a 686 nm interference filter, was detected by a photomultiplier coupled to a lock-in amplifier operating at 110 Hz. Other details are in the text.

were excited with 435 nm light (slit width 10 nm) and at least two fluorescence spectra were taken for each sample, using a Perkin-Elmer MPF 44A spectrofluorimeter as described in Ref. 28. The emission slit width was 5 nm.

Envelope-free chloroplasts prepared from pea leaves as previously described [29] were resuspended in a medium containing 0.1 M sorbitol, 5 mM $MgCl_2$ and 1 mM Hepes (pH 7.5) and kept on ice as a concentrated stock. To investigate effects of protein phosphorylation on chlorophyll fluorescence an aliquot of the stock was diluted into a medium consisting of 0.1 M sorbitol, 10 mM KCl, 1 mM $MgCl_2$, 5 mM NaF, ±0.15 mM ATP, 0.5 μM nigericin, 0.5 μM valinomycin and 10 mM Hepes (pH 7.8, KOH). When ATP was present, an equimolar amount of $MgCl_2$ was added to maintain the free Mg^{2+} concentration at 1 mM. Chlorophyll concentration in the suspension was 10 μg/ml. Chlorophyll fluorescence from a suspension in a cuvette was excited by Light 2 (about 20 W/m^2) which also served to reduce plastoquinone and presumably induce phosphorylation of thylakoid proteins, in particular LHCP [17,20]. When phosphorylation was induced by the reduction of plastoquinone with NADPH in the dark, the above medium was supplemented by 0.5 mM NADPH, 9 μM ferredoxin, 2.5 mM glucose, 100 μg/ml glucose oxidase and 200 μg/ml catalase. Chlorophyll fluorescence spectra at 77 K of chloroplast samples were made as for leaf samples (10 μg Chl/ml).

The relative degree of thylakoid membrane stacking was monitored by the chlorophyll content of a 10 000 × *g* pellet following digitonin treatment of a chloroplast sample, as previously described [29,30]. The chloroplast suspension contained 100 μg Chl/ml, 0.1 M sorbitol, 10 mM KCl, 1.3 mM $MgCl_2$, 5 mM NaF, 0.3 mM ATP, 1 μM nigericin, 1 μM valinomycin, 10 mM Hepes (pH 7.8, KOH) and ±20 μM DCMU. After a given period of illumination of the stirred suspension with Light 2 (about 80 W/m^2), an aliquot was treated with digitonin [29]. Chl *a* and *b* concentrations were determined by the method of Arnon [31].

Results

In this study, the experiments were aimed at a comparison between the State 1–State 2 transition in pea and barley leaves on the one hand, and the ATP-induced decrease in chlorophyll fluorescence from isolated envelope-free chloroplasts on the other. A common feature of the effects of the two processes is the apparent occurrence of increased spillover of excitation energy from PS II to PS I, and thus measurements dealt mainly with changes in spillover.

Fig. 2A shows an experiment which demonstrates the State 1–State 2 transition and its reversal. A dark-treated pea leaf attached to the plant was initially exposed to modulated blue-green light (Light 2). Complex transients in the chlorophyll fluorescence yield known as the Kautsky effect (see Ref. 14) were observed in the first few minutes. They reflect a number of processes occurring during this period, including adjustment to the light which preferentially excites PS II. Such an adjustment involves increased spillover to PS I, resulting in optimized electron flow from PS II to PS I and a more oxidized state of Q, and the leaf is said to be in State 2. Therefore when non-modulated Light 1 (which preferentially excites PS I) was superimposed, there was very little effect of the additional light on the fluorescence yield, indicating that at the time of superimposing far-red illumination the rate of electron transport was at its optimum and not limited by excitation of PS I. However, if the excess Light 1 was left on for several minutes, State 1 was induced, resulting in decreased spillover. On turning off this far-red light the preferential excitation of PS II associated with decreased spillover led to a more reduced state of Q and hence a higher chlorophyll fluorescence yield. The subsequent transition to State 2 during continued illumination by Light 2 alone is also shown in Fig. 2A. Fig. 2B depicts the behaviour of chlorophyll fluorescence yield in pea leaves pre-illuminated with Light 1. In the presence of excess Light 1, the application of modulated Light 2 produced a relatively small variation in the chlorophyll fluorescence yield, as State 1 should have been maintained. On turning off Light 1, the transition from State 1 to State 2 occurred at both 19 and 6°C, though more slowly at the lower temperature. At 6°C, the State 1–State 2 transition was not yet complete after 17 min so that Q was not optimally oxidized.

To study the transition from State 1 to State 2 at 19 and 6°C more closely, the illuminated leaves

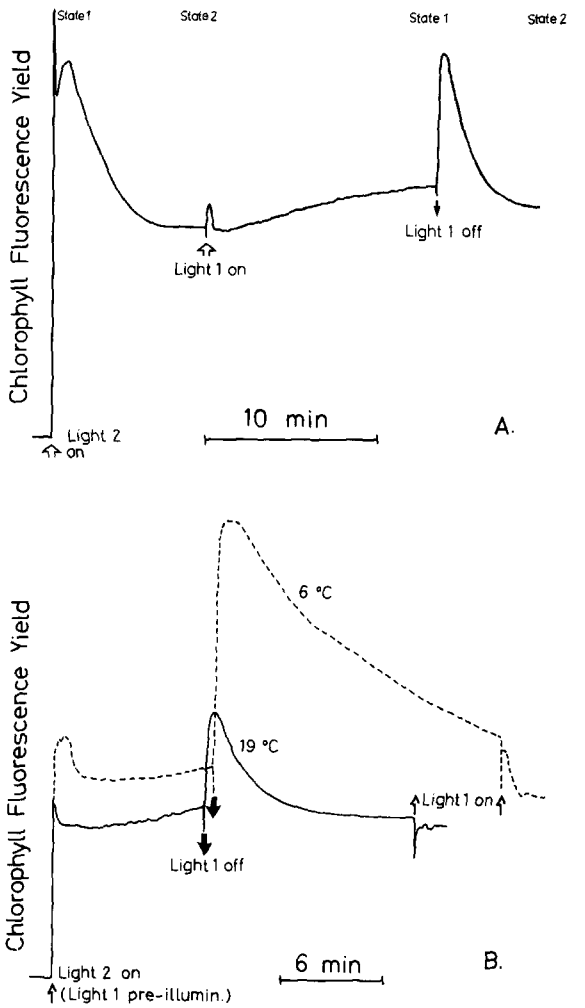

Fig. 2. Relative changes in the yield of modulated chlorophyll fluorescence from pea leaves attached to the parent plants. (A) The plant was dark pretreated for about 2 h. The initial effect of turning on the modulated Light 2 is to induce the Kautsky transients. After several minutes the leaf has adjusted to Light 2 (State 2) and the effect of superimposing excess Light 1 is initially small. However, treatment with both lights results in a slow transition from State 2 to State 1 as indicated by the ability of Light 2 to reduce Q when the supplementary Light 1 is turned off (observed as a rapid increase in the signal). The light conditions are as given in the legend of Fig. 1 and the experiment was done at room temperature. (B) Two pea leaves were pre-illuminated with Light 1 at 19°C for 10 min. One leaf was then exposed to Light 2 at 19°C while Light 1 was still on. The other leaf was further pre-illuminated with Light 1 for 10 min during the change from 19 to 6°C. It was then exposed to Light 2 at 6°C while Light 1 was still on. Subsequently, Light 1 was turned off and then on as shown by arrows.

were frozen in liquid nitrogen for measurement of chlorophyll fluorescence spectra. Qualitatively, the degree of spillover is taken to be related to F_{735}/F_{685}, the ratio of the fluorescence peak heights measured at 77 K [8,28,32,33], and is assumed to be unaffected by the freezing process. As can be seen in Table I, the transition from State 1 to State 2 seems to be accompanied by an increase in spillover, the change at 6°C over 15 min being smaller but nevertheless substantial.

Barley leaves attached to plants were also studied in relation to State 1–State 2 transitions. As shown in Fig. 3A, illumination of a dark-treated leaf of wild-type barley with Light 2 induced a Kautsky effect and State 2; the superposition of non-modulated Light 1 could reverse the transition, as was observed for a pea leaf in Fig. 2A. On the other hand, the Chl *b*-less mutant of barley exhibited no State 1–State 2 transition. Fig. 3B depicts the chlorophyll fluorescence yield changes when Light 1 and Light 2 were applied simultaneously; the Kautsky transients were relatively rapid (cf. Fig. 3A). Again, upon turning off Light 1, a State 1–State 2 transition is apparent in the wild type but not in the mutant.

TABLE I

THE TRANSITION FROM STATE 1 TO STATE 2 IN PEA LEAVES AT 19 AND 6°C

(a) Leaves were pre-illuminated with Light 1 for 10 min, followed by either 10 min of Light 1 to maintain State 1 or 10 min of Light 2 to induce State 2. (b) Leaves were pre-illuminated with Light 1 for 10 min at 19°C, then 10 min Light 1 during the change from 19 to 6°C, followed by either 15 min of Light 1 to maintain State 1 or 15 min of Light 2 to induce State 2 at 6°C. The leaves were subsequently frozen in liquid nitrogen for measurement of fluorescence spectra, as described in the text. Standard errors and numbers of replicates as indicated. Results in a and b were obtained on separate days.

Temperature		F_{735}/F_{685} (77 K)	Increase in F_{735}/F_{685} (State 1 → State 2)
(a) 19°C	State 1	7.22 ± 0.82 (4)	55.1%
	State 2	11.20 ± 1.19 (4)	
(b) 6°C	State 1	5.57 ± 0.25 (6)	30.6%
	State 2	7.51 ± 0.55 (7)	

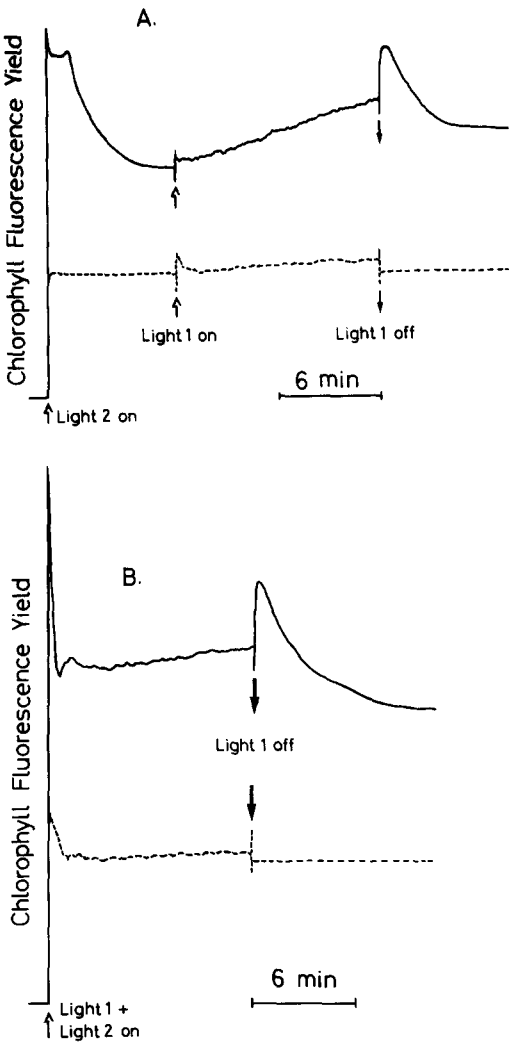

Fig. 3. Relative changes in the yield of modulated chlorophyll fluorescence from barley leaves attached to the parent plants at room temperature (approx. 23°C). The plants were dark pretreated for more than 1 h followed by the light regimes indicated in A and B. (———) Wild-type, (– – – –) mutant leaves. The light conditions are as given in the legend of Fig. 1.

Similar observations, not shown here, were also made when the intensities of Light 1 and Light 2 were increased to 12 and 7.5 W/m^2, respectively, at which the Kautsky transients became more apparent in both the wild type and mutant.

Since the barley mutant lacks Chl *b*, the absence of a State 1–State 2 transition is consistent with the concept that this transition involves the phosphorylation of LHCP [16–20]. To investigate this further, ATP-induced chlorophyll fluorescence quenching studies were conducted with isolated, envelope-free pea chloroplasts using Light 2 (approx. 20 W/m^2). Fig. 4A shows that the level of chlorophyll fluorescence decreased as the result of phosphorylation of LHCP, consistent with increased spillover, and in line with the observations and arguments of others [16–20]. The percentage of quenching of chlorophyll fluorescence for a given period of illumination decreased somewhat if the $MgCl_2$ concentration was

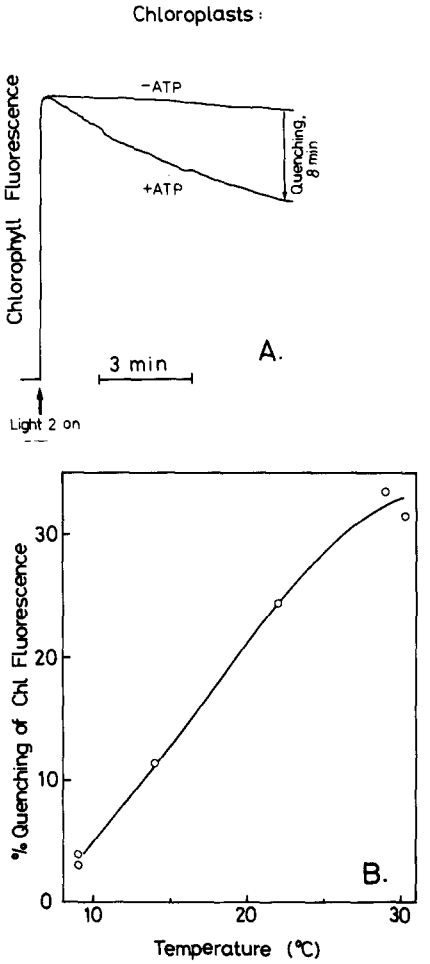

Fig. 4. ATP-induced quenching of chlorophyll fluorescence from isolated chloroplasts illuminated by Light 2 (approx. 20 W/m^2). The medium is described in the text. (A) An example of the quenching as illumination proceeded at 29°C. (B) The % quenching after 8 min of illumination is depicted for several temperatures.

increased, for example, to 2 or 5 mM. The temperature sensitivity of the fluorescence quenching over a given period of illumination (8 min) is displayed in Fig. 4B; there is an approx. 10-fold decrease in the ATP effect on lowering the temperature from 29 to 9°C. Using a separate preparation of chloroplasts, the increase in spillover thought to be associated with phosphorylation of LHCP was further studied at 19 and 4.5°C. Table II shows that after 15 min of light treatment, the quenching of chlorophyll fluorescence was only 4.3% at the lower temperature. In contrast, a pea leaf at a comparable temperature (6°C as measured by a thermocouple resting on the upper surface of a leaf cooled from below) seemed to exhibit a more substantial increase in spillover, as seen in Fig. 2B and Table I.

Since phosphorylation of LHCP is believed to be controlled by the redox state of plastoquinone [17–20], it should be possible to reduce plastoquinone not only with light as demonstrated above, but also chemically. Such experiments have been done using redox mediators [18–20] but here we have used DCMU-treated envelope-free chloroplasts incubated with NADPH, ferredoxin and ATP in the dark for 20 min at room temperature. Under these conditions substantial quenching of the chlorophyll fluorescence was observed (47%); the control and quenched samples when frozen in liquid nitrogen exhibited the fluorescence spectra in Fig. 5, showing an increased F_{735}/F_{685} ratio indicative of an increase in spillover with ATP-treated samples.

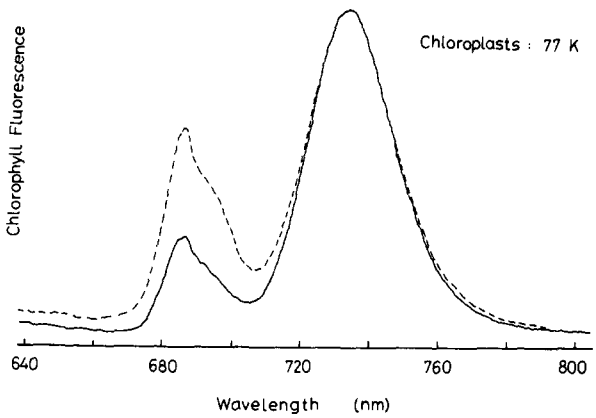

Fig. 5. Chlorophyll fuorescence spectra of isolated chloroplasts at 77 K, measured in the presence of 20 μM DCMU. Chloroplasts were pretreated for 20 min with 0.5 mM NADPH and 9 μM ferredoxin, with (———) or without (– – – –) 0.15 mM ATP, so as to reduce plastoquinone in the dark. Other constituents in the medium are given in the text. At the end of the incubation period, aliquots were frozen in liquid nitrogen. The fluorescence spectra are normalized at the long-wavelength peak.

An experiment to investigate a possible connection between ATP-induced spillover and thylakoid membrane stacking was attempted. The relative degree of thylakoid stacking was monitored during illumination of a chloroplast sample in the presence of ATP. The method involves the use of a non-ionic detergent, digitonin, which is assumed to disrupt thylakoid membranes to an extent dependent upon

TABLE II

ATP-INDUCED QUENCHING OF CHLOROPHYLL FLUORESCENCE FROM PEA CHLOROPLASTS AT 19 AND 4.5°C, AND ASSOCIATED CHANGES OF F_{735}/F_{685} MEASURED AT 77 K

Envelope-free chloroplasts were pre-incubated at the required temperature for 5 min and then illuminated with Light 2 (about 20 W/m^2) for 15 min. The chlorophyll fluorescence level was noted at the end of 15 min of Light 2, and an aliquot of chloroplast suspension was frozen in liquid nitrogen for measurement of F_{735}/F_{685}. The medium is described in the text. Values are means of duplicates, except in one case where there were four replicates.

Temperature		% Quenching of chlorophyll fluorescence	F_{735}/F_{685} (77 K)	ATP-induced increase in F_{735}/F_{685}
19°C	+ATP	39.5	2.51	67.3%
	–ATP		1.50	
4.5°C	+ATP	4.3 ± 0.6 (4)	1.46	11.5%
	–ATP		1.31	

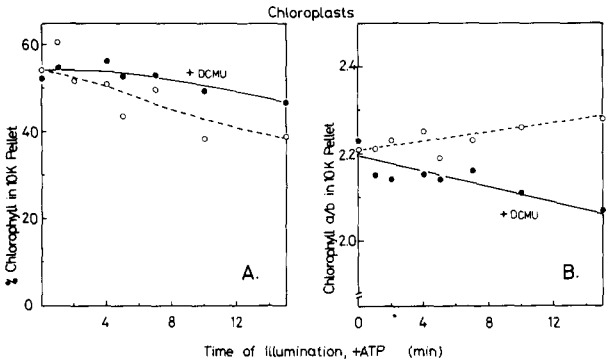

Fig. 6. Structural changes in isolated chloroplasts resulting from illumination with Light 2 (approx. 80 W/m^2) in the presence of 0.3 mM ATP, as monitored by the use of digitonin to disrupt thylakoid membranes. Samples treated with 20 μM DCMU so as to inhibit the ATP effect served as controls. Other conditions are described in the text. (A) The amount of chlorophyll in the 10 000 $\times g$ pellet gives a relative measure of the degree of stacking. Completely unstacked thylakoids typically gave about 10% Chl in the '10K' pellet. (B) The Chl *a/b* ratio in the '10K' pellet gives a measure of the randomization of the photosystems before digitonin treatment. The ratio for a pellet derived from completely unstacked thylakoid membranes is similar to the Chl *a/b* ratio of whole chloroplasts (typically 3.0).

the degree of thylakoid stacking [29,30,42]. The results are shown in Fig. 6A, where control samples are those in which DCMU prevented phosphorylation of LHCP. It is seen that phosphorylation of LHCP led to some decrease in the degree of thylakoid stacking as measured by the chlorophyll content of the heavy fraction (10K pellet). Fig. 6B shows that the Chl *a/b* ratio in the 10K pellet increased with the extent of phosphorylation of LHCP as compared with the control samples in the presence of DCMU.

Discussion

In this paper chlorophyll fluorescence has been used to monitor indirectly changes in the relative quantum efficiencies of PS II and PS I in intact leaves and in isolated pea chloroplasts which have been incubated with ATP under conditions which lead to the phosphorylation of LHCP. In the case of intact leaves, continuous illumination with Light 2 seems to result in an increase in spillover from PS II to PS I so that Q becomes more oxidized as judged by using additional PS I light as a probe of the redox state of the PS II reaction centres. This condition is termed State 2 [5,14]. State 1 is achieved by a long dark pretreatment period of the leaf or by illuminating it for several minutes with excess PS I light. After these treatments, spillover from PS II to PS I is at a minimum so that Light 2 preferentially excites PS II and Q is in a more reduced state. It seems that the fluorescence decrease due to ATP addition to isolated chloroplasts can be identified with the State 1–State 2 changes based on the comparison of the F_{735}/F_{685} ratios measured at 77 K. Despite possible artefacts in the method of freezing samples in liquid nitrogen [33], a higher ratio of F_{735}/F_{685} was consistently found for phosphorylated chloroplasts (Table II), in agreement with previous reports [16, 19], and for leaf samples brought to State 2 as compared with those in State 1 (Table I). The high ratios obtained for leaves (compared with values for isolated chloroplasts, Table II) were due to self-absorption of the 685 nm peak at the high chlorophyll content of leaf material.

Further support that the in vivo State 1–State 2 change may be related to the phosphorylation process comes from the studies with the barley mutant lacking Chl *b* and LHCP. This mutant exhibited neither an ATP-induced decrease in chlorophyll fluorescence of the isolated chloroplasts (data not shown) nor a State 1–State 2 transition in leaves. These results also implicate LHCP in both phenomena as suggested by Bennett et al. [16]. It remains to be determined as to what takes the place of LHCP in organisms such as red algae which lack LHCP but can nevertheless undergo State 1–State 2 transitions, even relatively rapidly and with low quantum requirements [9–11].

Changes in the redox state of plastoquinone have been found to accompany the State 1–State 2 transition in bean leaves [34]. There is also convincing evidence that the redox state of plastoquinone controls protein phosphorylation in isolated chloroplasts [17,18] and the accompanying quenching of chlorophyll fluorescence [11,19]. The results in Fig. 5 give further evidence that reduction of plastoquinone in the dark by NADPH [35] also leads to increased spillover in DCMU-treated isolated chloroplasts, consistent with the finding [24] that protein kinase activity in the dark can be increased by NADPH plus ferredoxin. Thus, the evidence is strong for the con-

cept that the redox state of plastoquinone in both leaves and isolated chloroplasts can control spillover in such a way as to optimize electron transport via PS II and PS I under light-limiting conditions [17, 20].

The manner in which spillover from PS II to PS I is increased by protein phosphorylation is as yet unclear. The results in Fig. 6A suggest that protein phosphorylation in isolated chloroplasts led to a slight decrease in the degree of stacking. This result is consistent with the finding that the degree of thylakoid stacking in whole *Chlamydomonas reinhardii* cells changed from 60% in State 1 to 40% in State 2 [7]. Our finding that the Chl *a/b* ratio in the digitonin-derived heavy fraction increased after incubation with ATP (Fig. 6B) suggests that there had been some randomization of the PS I and PS II complexes as a result of the phosphorylation. Such a randomization of fluorescing PS II/LHCP complexes and quenching PS I centres would be expected to give a lower chlorophyll fluorescence yield due to increased spillover and a decrease in the degree of thylakoid stacking [36].

The cause of the slight randomization of the PS II and PS I complexes associated with protein phosphorylation has yet to be fully elucidated. The additional negative charge introduced onto the thylakoid membrane surface as a result of covalent binding of the phosphate groups to proteins would seem to represent only a small fraction of the net negative charge density (σ) that is already present. Taking the amount of covalently bonded phosphate to be 4 nmol/mg Chl [16], an area of $2.6 \cdot 10^{-3}$ m^2/thylakoid [37] and 10^5 Chl molecules/thylakoid [38], one obtains an estimate of $2.2 \cdot 10^{-4}$ C/m^2 assuming each phosphate group carries one negative charge. This is only about 1% of $2 \cdot 10^{-2}$ C/m^2, a plausible average σ value for thylakoids [39]. Indeed, experimental attempts to determine σ for thylakoids before and after protein phosphorylation using the method of 9-aminoacridine fluorescence quencing [39] yielded no detectable difference between the two types of chloroplasts. On the other hand, the distribution of surface charges on thylakoid membranes is likely to be heterogeneous [40,41], stacking occurring between adjacent membranes only where the electrostatic repulsion is weak because of a low level of surface charge, and where the van der Waal's attraction is high because of a high protein density, e.g., in domains of PS II/LHCP [36,41–43]. In this case, the localized injection of negative charge due to phosporylation of some of the LHCP could be sufficiently significant to cause unstacking to some extent, for example, at the periphery of the grana [36]. Consistent with this electrostatic model is the finding, reported above, that the ATP-induced quenching of chlorophyll fluorescence was somewhat inhibited when the $MgCl_2$ concentration was increased to 2 or 5 mM, corresponding to increasing electrostatic screening.

At low temperature (4.5–6°C) the transition from State 1 to State 2 (Table I) in leaves seemed to occur much more readily than the ATP-induced quenching of chlorophyll fluorescence from isolated chloroplasts (Table II and Fig. 4B). This lack of correlation between the two phenomena could be explained if, after isolation of chloroplasts, the presently unknown link between the redox state of plastoquionone and protein kinase, or the kinase activity itself, becomes more temperature sensitive. Indeed, it is usually found that 2 h after isolation of envelope-free chloroplasts, the ATP effect on chlorophyll fluorescence is substantially lost, emphasizing the delicate nature of the enzyme system.

In conclusion, leaves of higher plants show State 1–State 2 changes similar to those reported in algae. The mechanism responsible for the regulation of quantal input to PS I and PS II seems to require the presence of LHCP and can be identified with the phosphorylation/dephosphorylation of this protein. Changes in the surface charge density of the exposed segment of LHCP could be sufficient to cause partial unstacking and randomization of some PS II/LHCP with PS I pigment proteins in unstacked membranes in such a way as to increase energy transfer from PS II to PS I.

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