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Pigment orientation changes accompanying the light state transition in *Synechococcus* sp. PCC 6301

Jill A. Homer-Dixon[1], Elisabeth Gantt[2] and Doug Bruce[1,*]
[1]*Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada, L2S 3A1;* [2]*Department of Botany and Maryland Agricultural Experiment Station, University of Maryland, College Park, MD 20742, USA; * Author for correspondence*

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Abstract

Low temperature (77 K) linear dichroism spectroscopy was used to characterize pigment orientation changes accompanying the light state transition in the cyanobacterium, *Synechococcus* sp. PCC 6301 and those accompanying chromatic acclimation in *Porphyridium cruentum* in samples stabilized by glutaraldehyde fixation. In light state 2 compared to light state 1 intact cells of *Synechococcus* showed an increased alignment of allophycocyanin parallel to the cells' long axis whereas the phycobilisome-thylakoid membrane fragments exhibited an increased allophycocyanin alignment parallel to the membrane plane. The phycobilisome-thylakoid membrane fragments showed less alignment of a short wavelength chlorophyll *a* (Chl *a*) Q_y transition dipole parallel to the membrane plane in state 2 relative to state 1.

To aid identification of the observed Chl *a* orientation changes in *Synechococcus*, linear dichroism spectra were obtained from phycobilisome-thylakoid membrane fragments isolated from red light-grown (increased number of PS II centres) and green light-grown (increased number of PS I centres) cells of the red alga *Porphyridium cruentum*. An increased contribution of short wavelength Chl *a* Q_y transition dipoles parallel to the long axis of the membrane plane was directly correlated with increased levels of PS II centres in red light-grown *P. cruentum*.

Our results indicate that the transition to state 2 in cyanobacteria is accompanied by an increase in the orientation of allophycocyanin and a decrease in the orientation of Chl *a* associated with PS II with respect to the thylakoid membrane plane.

Abbreviations: APC – allophycocyanin; Chl *a* – chlorophyll *a*; DCMU – 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; LD – linear dichroism; LD/A – linear dichroism divided by absorbance; LHC – light-harvesting complex; PBS – phycobilisome; PC – phycocyanin; PS – Photosystem

Introduction

State transitions

Photosynthetic organisms containing the chlorophyll *a/b* light harvesting complex II (LHC II Chl *a/b*) or phycobilisomes (PBS) as the major light-harvesting antenna have been proposed to regulate the distribution of excitation energy between PS II and PS I through the light state transition (Bonaventura and Myers 1969, Murata 1969). The state transition is believed to balance PS I

and PS II activities in response to preferential excitation of either photosystem.

The generally accepted mechanism of the state transition in the LHCII-containing higher plants involves reversible phosphorylation of the LHCII Chl *a/b* complex and its consequent lateral migration between grana thylakoid membranes, rich in PS II, and stroma thylakoid membranes, rich in PS I (Barber 1986, Williams and Allen 1987).

A number of models for the mechanism of the light state transition in the PBS containing cyanobacteria and red algae have been proposed. In the original 'spillover' model (Murata 1969, reviewed in Biggins and Bruce 1989), excitation energy distribution is proposed to be regulated through membrane conformational changes that increase the probability of energy transfer from PS II Chl *a* antenna to PS I Chl *a* antenna. The 'mobile antenna' model proposes phosphorylation of the PBS, dissociation of the phospho-PBS from PS II and its subsequent association with PS I in a mechanism which parallels that of LHC II containing organisms (Allen et al. 1985, Sanders et al. 1986). A modified form of this model, the 'PBS-detachment' model, has also been proposed in which energetic dissociation of the PBS from PS II follows PBS phosphorylation but there is no subsequent association of the phospho-PBS with PS I (Mullineaux and Allen 1988). Recent work has made it evident that none of the above models sufficiently recognizes and encompasses the present experimental results and that the mechanism of the state transition in cyanobacteria and red algae remains enigmatic (Mullineaux and Holzwarth 1991, Mullineaux et al 1991, Mullineaux 1992, Bruce and Salehian 1992, Salehian and Bruce 1992).

Pigment orientation in cyanobacteria

Information about pigment orientation and membrane conformation associated with the two light states in PBS containing organisms has been sought to help determine the molecular mechanism behind the observed changes in the distribution of energy between PS II and PS I. Freeze-fracture electron microscopy has shown the exoplasmic fracture face of the state 1 thylakoid membrane in the cyanobacteria *Synechocystis* sp. and a phycocyanin- (PC-) deficient mutant thereof to be characterized by rows of PS II particles (Olive et al. 1986, Vernotte et al. 1990). State 2 thylakoid membranes are characterized by a somewhat more random arrangement of exoplasmic face particles. Methods in polarized light spectroscopy have also been employed to identify pigment orientation differences between oriented thylakoids cross-linked with glutaraldehyde in the two light states. Bruce and Biggins (1985), using 77 K linear dichroism spectroscopy, identified a relative increase in absorption of allophycocyanin core pigments parallel to the long axis of intact cells of the cyanobacterium *Synechococcus* sp. PCC 6301 fixed with glutaraldehyde in state 2. The lack of a state transition competent PBS-thylakoid membrane preparation made studies of pigment orientation with respect to the thylakoid membrane plane impossible until the development of a technique to isolate stabilized PBS-thylakoid membrane fragments from intact cyanobacteria chemically cross-linked in state 1 or state 2 with glutaraldehyde (Brimble and Bruce 1989). The isolated PBS-thylakoid membrane preparations maintained the 77 K fluorescence emission characteristics of state 1 or state 2 and allowed the detection of differences in Chl *a* orientation with respect to the thylakoid membrane plane (Brimble and Bruce 1989). The room temperature linear dichroism spectra of that study showed no distinctive re-orientation of phycobilin in the PBS-thylakoid membrane fragments.

In this report, low temperature (77 K) linear dichroism spectroscopy has been employed to more rigourously investigate pigment orientation differences in intact cells and PBS-thylakoid membrane fragments from *Synechococcus* sp. 6301 fixed with glutaraldehyde in the two light states. Cells and membrane fragments in state 2 were characterized by increased orientation of allophycocyanin transition dipoles parallel to the long axis of the cell and parallel to the membrane plane, respectively. In addition state 2 PBS-thylakoid membrane fragments were also characterized by a decrease in the orientation of short

wavelength Chl *a* Q_y transition dipoles parallel to the membrane plane. Identification of the short wavelength Chl *a* as PS II associated was substantiated by comparison of the LD spectra of PS II rich and poor PBS-thylakoid membrane fragments isolated from cells of the red algae *P. cruentum* grown under red light and green light, respectively.

Materials and methods

Cyanobacterial and red algal cultures

The cyanobacterium *Synechococcus* sp. PCC 6301 was grown photoautotrophically in 200 ml batch cultures at 30 °C, in BG-11 medium, at a light intensity of 25 μE m^{-2} s^{-1} as described in Brimble and Bruce (1989). *Porphyridium cruentum* was grown photoautotrophically at 15 μE m^{-2} s^{-1} in Jones medium and harvested as in Cunningham et al. (1990). One litre batch cultures were grown at 18 °C and bubbled with 5% CO_2. Cells were cultured under red or green light provided by Sylvania fluorescent tubes filtered by Roscolux No. 27 medium red, or P-40 dark green coloured plastic sheets, respectively, and harvested during the exponential growth phase.

Sample preparation

Cyanobacterial cells were harvested in the exponential growth phase and resuspended at a concentration of 2 μg Chl ml^{-1} in 45 ml of growth media containing 4 μM DCMU. Cells were brought to state 1 by oxidation of the plastoquinone pool induced by 5 min illumination with 30 μE m^{-2} s^{-1} of white light and to state 2 by reduction of the plastoquinone pool induced by a 5 min dark incubation. After the induction period for either state, glutaraldehyde was added to the constantly stirred cells to a final concentration of 1%, cells were then kept under the state 1 or state 2 induction conditions for a further 5 min. The glutaraldehyde fixed cell suspensions were diluted to four times their volume in buffer (10 mM Tricine, pH 7.5 containing 100 mM sucrose) and fixed cells isolated by centrifugation at 3000 g for 5 min. The cells were washed once and resuspended in 10 ml of buffer in preparation for membrane isolation, or washed and resuspended in a minimal volume of buffer in preparation for LD spectroscopy.

PBS-thylakoid membrane fragments were isolated from both *S.* sp. PCC 6301 and *P. cruentum* after glutaraldehyde fixation by breakage in a French pressure cell (16,000 psi), removal of large fragments (1000 g, 10 min), supernatent layering on a sucrose gradient (0.2–1.75 M) and collecting phycobilin-chlorophyll samples from the 0.5 M (*Synechococcus*) or 1.75 M (*Porphyridium*) layer following centrifugation (50,000 g, 6 h) as described in Brimble and Bruce (1989).

Samples were oriented for LD spectroscopy using the gel squeezing method (Adbourakhmanov et al. 1979, Brimble and Bruce 1989). Gels were composed of a 10 mM Tricine solution (pH 7.5) containing 2.2 M sucrose, 10% acrylamide and sample to a final concentration of 5 μg Chl ml^{-1}. Polymerization proceeded on ice in the dark.

Spectroscopy

Linear dichroism spectroscopy was performed using the laboratory-built apparatus described by Brimble and Bruce (1989). Linear dichroism was measured as $A_{parallel}$-$A_{perpendicular}$, where $A_{parallel}$ represents the absorption of light polarized parallel to the long axis of the oriented sample (the direction of gel expansion) and $A_{perpendicular}$ represents the absorption perpendicular to this axis. The LD apparatus was designed to allow simultaneous collection of absorbance spectra (Brimble and Bruce, 1989). Absorbance corrected LD spectra or 'reduced' LD spectra (LD/A) were calculated by dividing the raw LD spectrum by its corresponding absorbance spectrum. Absorbance, LD and LD/A spectra presented here are the averages of spectra from at least three different squeezed gels made from independent sample preparations.

77 K fluorescence emission spectra from samples resuspended in polymerized polyacrylamide/sucrose gels were obtained using the laboratory built fluorimeter described by Brimble and Bruce (1989).

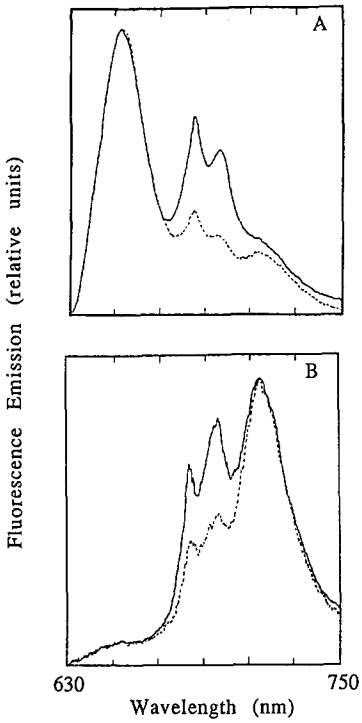

Fig. 1. 77 K fluorescence emission spectra of intact cells of *Synechococcus* sp. PCC 6301. Cells were cross-linked with glutaraldehyde in state 1 (solid trace) and state 2 (broken trace) and suspended in a polyacrylamide/sucrose gel (see Methods). In panel A the excitation wavelength was 590 nm and the spectra were normalized at 653 nm. In panel B the excitation wavelength was 435 nm and the spectra were normalized at 715 nm.

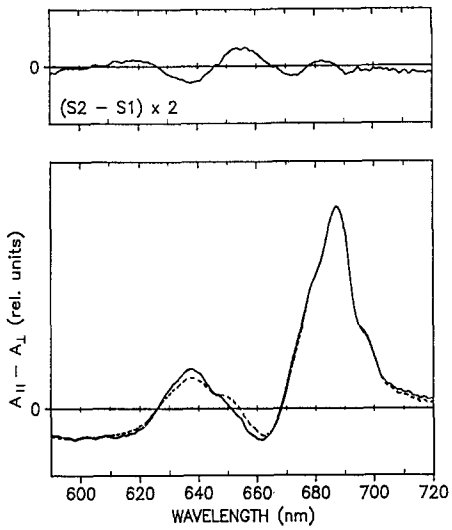

Fig. 2. 77 K linear dichroism ($A_{parallel}$ - $A_{perpendicular}$) of intact cells of *Synechococcus* sp. PCC 6301. Cells were cross-linked with glutaraldehyde in state 1 (solid trace) and state 2 (broken trace) and oriented in a squeezed polyacrylamide/sucrose gel (see Methods). The state 2 minus state 1 difference spectrum is shown in the upper panel.

Results

Intact cells

Figure 1 compares the 77 K fluorescence emission spectra of intact cells of *Synechococcus* sp. PCC 6301 cross-linked with glutaraldehyde in state 1 and state 2 and incorporated into a polyacrylamide/sucrose gel. Figure 1A shows emission peaks resulting from a 590 nm actinic beam, chosen to excite phycocyanin (PC). The 685 nm and 695 nm PS II associated Chl *a* emission peaks were higher in the state 1 spectrum relative to the 715 nm PS I Chl *a* emission peak following normalization at the 653 nm PC peak emission. This observation was consistent with differences in emission characteristics between state 1 and state 2 (Murata 1969, Ley and Butler 1976, Brimble and Bruce 1989, Salehian and Bruce 1992). Figure 1B shows emission resulting from excitation of Chl *a* at 435 nm. Again, in state 1 emission from the 685 nm and 695 nm PS II peaks was greater relative to the 715 nm PS I emission maximum. As relative differences in fluorescence emission indicative of state 1 and state 2 are observed in intact cells, we conclude that the energy transfer characteristics within the thylakoid membranes have not been affected by incorporation of cells into the gel.

Linear dichroism (LD) spectra of *Synechococcus* cells cross-linked with glutaraldehyde in state 1 and state 2 are presented along with the state 2 minus state 1 difference spectrum in Fig. 2. Such spectra exhibit a strong positive LD band at 688 nm, with shoulders at 679 nm and 698 nm, representing Chl *a* Q_y transitions. The positive LD signal showed these transitions to be preferentially oriented more parallel to the long axis of the cell (Bruce and Biggins 1985). The phycobilin absorption region of the LD spectra was characterized by a positive PC peak at 638 nm, and an allophycocyanin (APC) shoulder at 651 nm.

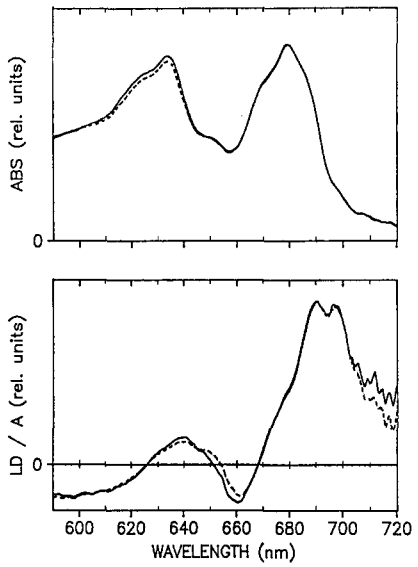

Fig. 3. 77 K absorbance spectra and absorbance corrected linear dichroism spectra (LD/A) of intact cells of *Synechococcus* sp. PCC 6301. Cells were cross-linked with glutaraldehyde in state 1 (solid traces) and state 2 (broken traces) and oriented in a squeezed polyacrylamide/sucrose gel (see Methods). The upper panel contains the absorbance spectra (ABS) and the lower panel the absorbance corrected linear dichroism spectra (LD/A).

The state 2 minus state 1 LD difference spectrum (Fig. 2) had a negative peak at 638 nm, indicating that the PC transition dipoles were oriented relatively less parallel to the long axis of the intact cell in state 2 as compared to state 1. In the APC-absorbing region, a positive 656 nm peak in the difference spectrum showed that the transition dipole of this PBS core pigment was oriented more parallel to the long axis of the intact cell in state 2 than in state 1. This latter finding confirmed earlier results obtained from 77 K (Bruce and Biggins 1985) and room temperature (Brimble and Bruce 1989) LD of intact cells. The LD spectra showed little difference in Chl *a* orientation between the two light states, however the difference spectrum featured a small negative contribution around 675 nm which was also present in the room temperature spectra reported by Brimble and Bruce (1989).

In the absorbance spectra for intact cells in state 1 and state 2, in the upper panel of Fig. 3, two PC absorption bands are indicated by the 634 nm peak and 625 nm shoulder. There was only a small difference in PC absorption, and in the 652 nm shoulder of APC, between the two samples. Chl *a* Q_y transitions were indicated by the 679 nm peak with shoulders at 669 nm, 686 nm and 695 nm.

Peaks in raw LD spectra are indicative of both the amount of chromophore and the orientation of the transition dipole. Absorbance corrected linear dichroism spectra (LD/A) allow comparisons of chromophore orientation independent of chromophore content. LD/A spectra of cells cross-linked in both light states are presented in the lower panel of Fig. 3. In the Chl *a* Q_y region peaks were observed at 688 nm and 697 nm with shoulders at 679 nm and 670 nm. The LD/A spectra showed that the long wavelength transitions at 688 nm and 697 nm were more highly oriented than those at 679 nm and 670 nm. The small negative peak at 675 nm observed in the LD state 2 minus state 1 difference spectrum (Fig. 2) was retained but red shifted to about 680 nm in the LD/A state 2 minus state 1 difference spectrum (not shown). No other significant differences between LD/A spectra of intact cells in state 1 and state 2 in the Chl *a* region were observed. The differences observed at wavelengths longer than 700 nm were not considered significant as small changes in the very low absorption in this region have dramatic effects on LD/A.

In the phycobilin region the differences between cells in state 1 and state 2 observed in the raw LD spectra (Fig. 2) were also retained in the LD/A spectra (Fig. 3). Thus PC transition dipoles were less aligned with the long axis of the cell, and APC transition dipoles more aligned in state 2 than state 1.

PBS-thylakoid membrane fragments

The PBS-thylakoid membranes retained the excitation energy distribution patterns characteristic of each state as seen by the fluorescence emission spectra of PBS-thylakoid membrane fragments isolated from *Synechococcus* cross-linked with glutaraldehyde in each state and suspended in acrylamide/sucrose gel (Fig. 4). With both 590 nm (Fig. 4A) and 435 nm (Fig. 4B) exci-

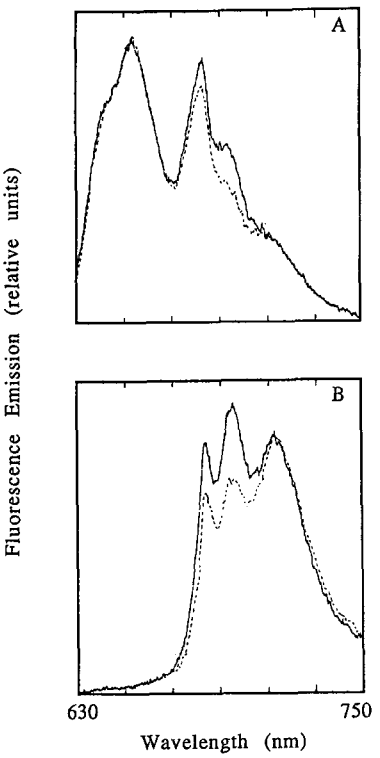

Fig. 4. 77 K fluorescence emission spectra of PBS-thylakoid membrane fragments isolated from *Synechococcus* sp. PCC 6301. The fragments were isolated from cells cross-linked with glutaraldehyde in state 1 (solid traces) and state 2 (broken traces) and were suspended in polyacrylamide/sucrose gel (see Methods). In panel A the excitation wavelength was 590 nm and the spectra were normalized at 653 nm. In panel B the excitation wavelength was 435 nm and the spectra were normalized at 715 nm.

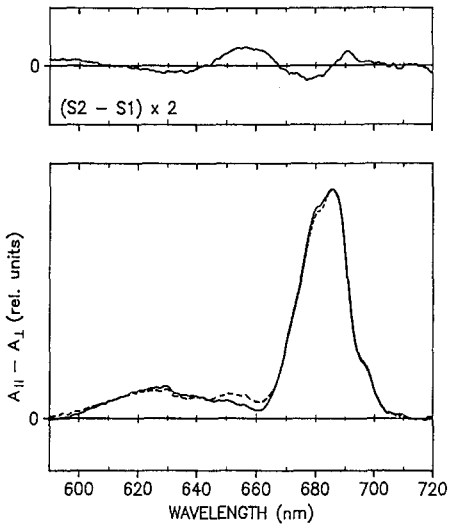

Fig. 5. 77 K linear dichroism of PBS-thylakoid membrane fragments isolated from *Synechococcus* sp. PCC 6301. The fragments were isolated from cells cross-linked with glutaraldehyde in state 1 (solid trace) and state 2 (broken trace) and were then oriented in a squeezed polyacrylamide/sucrose gel (see Methods). The state 2 minus state 1 difference spectrum is shown in the upper panel.

tation, the fluorescence emission at 685 nm and 695 nm (PS II associated) was greater than that at 715 nm (PS I associated) in state 1, as previously observed in PBS-thylakoid membrane fragments by Brimble and Bruce (1989). The emission spectra from PBS-thylakoid membrane fragments showed some changes from those of intact cells (most notably increased phycobilin and PS II Chl *a* fluorescence yields as compared to PS I Chl *a* emission). The relative increase in phycobilin fluorescence most likely arises from less efficient energetic coupling of phycobilin to Chl *a* and/or the presence of a small amount of free phycobilin. The relative increase in PS II emission may reflect fluorescence yield changes of either photosystem or a slight enrichment of PS II in the preparations as compared to intact cells.

Figure 5 presents the 77 K LD and LD difference spectra for the isolated PBS-thylakoid membrane fragments. The contribution of the phycobilin was decreased compared to the whole cell spectra (Fig. 1). A broad PC peak was located at 630 nm, while the APC positive maximum was found at 655 nm. The Chl *a* Q_y transition peak was at 686 nm, with shoulders at 680 nm and 697 nm.

As in intact cells, the LD spectra of PBS-thylakoid membrane fragments showed PC chromophores to be slightly less dichroic, and APC chromophores more dichroic in state 2 compared with state 1 (Fig. 5). In the state 2 minus state 1 difference spectrum of Fig. 5 the negative PC maximum was slightly red shifted (to approximately 630 nm) relative to that for intact cells although the positive APC band remained at 656 nm.

Small differences in dichroism in the Chl *a* Q_y region were apparent in the LD spectra of PBS-thylakoid membrane fragments between states. The state 2 minus state 1 difference spectrum had

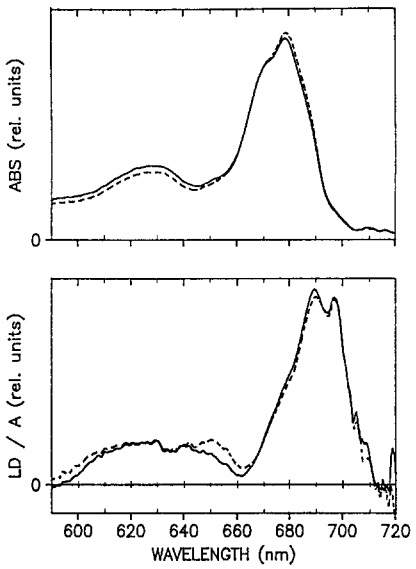

Fig. 6. 77 K absorbance spectra and absorbance corrected linear dichroism spectra (LD/A) of PBS-thylakoid membrane fragments isolated from *Synechococcus* sp. PCC 6301. The fragments were isolated from cells cross-linked with glutaraldehyde in state 1 (solid traces) and state 2 (broken traces) and were then oriented in a squeezed polyacrylamide/sucrose gel (see Methods). The upper panel contains the absorbance spectra (ABS) and the lower panel the absorbance corrected linear dichroism spectra (LD/A).

a negative 677 nm peak and a small positive 691 nm peak (Fig. 5, upper panel). These features in the state 2 minus state 1 difference spectrum were similar to those previously observed (negative 675 nm peak and positive 692 nm peak) in a room temperature LD difference spectrum of isolated PBS-thylakoid membrane fragments (Brimble and Bruce 1989).

The 77 K absorbance spectra of PBS-thylakoid membrane fragments (Fig. 6) showed a significantly decreased contribution by PC relative to that of intact cells, presumably due to some dissociation of PBS rods and loss of PC during isolation. By comparison of absorption spectra we estimate that approximately 80% of PC, and less than 20% of APC is lost during the isolation procedure (data not shown). There were also some differences in PC absorption between state 1 and state 2, most likely reflecting different amounts of PC remaining in the two samples of isolated membrane. A slight difference in the absorption at the 679 nm peak was also noted.

The above mentioned differences in absorption may have contributed to the differences observed in the LD spectra of Fig. 5. Absorbance corrected LD/A spectra were again calculated (Fig. 6) to distinguish between differences in chromophore orientation and chromophore content. The difference in PC dichroism observed in the LD spectra of Fig. 5 was lost after correction for absorption, however the increased dichroism in the APC region (656 nm) in state 2 remained. The increased dichroism in state 2 is thus regarded as being indicative of increased orientation of an APC transition parallel to the membrane plane.

As was the case for intact cells, the LD/A spectra of PBS-thylakoid membrane fragments in Fig. 6 showed the long wavelength Chl *a* transitions at 688 nm and 697 nm to be more highly oriented than the shorter wavelength Chl *a* transitions. The LD/A spectra also retained the decrease in short wavelength Chl *a* dichroism in state 2 observed in the raw LD spectra (negative 679 nm peak in Fig. 5) although the negative peak in the LD/A difference spectrum was red shifted to about 682 nm (not shown). These results confirm a decrease in orientation of a short wavelength Chl *a* Q_y transition parallel to the membrane plane in state 2.

Chromatic acclimation in P. cruentum: Identification of PS II and PS I associated Chl a *Q_y transitions*

In order to further identify the Chl *a* transitions observed in PBS- thylakoid membrane fragments from *Synechococcus*, LD spectra were made from PBS-thylakoid membrane fragments isolated from *Porphyridium* enriched in PS II and PS I (Fig. 7). Thylakoids of red light-grown *Porphyridium* cells contain more PS II and less PS I centres (PS II/PS I of 1.21) than those of green light-grown cells (PS II/PS I of 0.26) (Cunningham et al. 1990). PBS-thylakoid membrane fragments with the higher PS II content showed a greater contribution by shorter wavelength Chl *a* in the Q_y region (the 679 nm peak and the 671 nm shoulder) than fragments with lower PS II content. On the long wavelength side of the Chl *a*

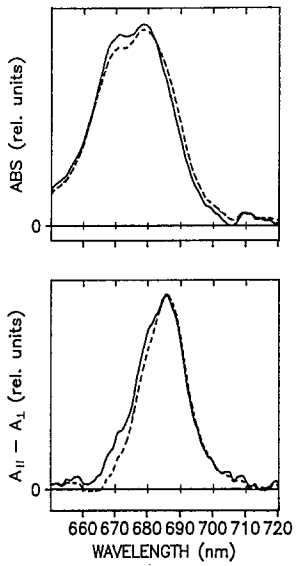

Fig. 7. 77 K absorption and linear dichroism of PBS-thylakoid membrane fragments isolated from *Porphyridium cruentum*. Fragments were isolated from red light-grown (solid traces) and green light-grown (broken traces) cells cross-linked with glutaraldehyde. The upper panel contains the absorbance spectra (ABS) and the lower panel the linear dichroism spectra.

Q_y transition, absorption was greater in fragments isolated from cells with a higher PS I content.

LD spectra of PBS-thylakoid membrane fragments with higher PS II content from *Porphyridium* also showed much greater dichroism on the short wavelength side of the Q_y transition peak, including a shoulder at 680 nm (Fig. 7). The Chl *a* peak from fragments isolated from green light-grown algae was slightly red-shifted with respect to that from fragments isolated from red light-grown thylakoids. The short wavelength oriented Chl *a* transitions at 670 nm and 680 nm in *Porphyridium* PBS-thylakoid membrane fragments were thus substantiated as PS II associated.

The absorbance corrected LD/A spectra of the PBS-thylakoid fragments isolated from red light and green light-grown cells of *Porphyridium* were not significantly different (not shown). This indicates that the observed changes in the LD spectra (Fig. 7) can be ascribed to differing amounts of PS II and PS I in green and red light-grown cells and not to differences in the orientation of the complexes in the membrane.

Discussion

Despite much investigation the mechanism of the light state transition in cyanobacteria remains enigmatic. Controversy about the mechanism has focused on the relative involvement of Chl *a* and phycobilin, the role of protein phosphorylation and on whether the state transition serves to regulate the distribution of excitation energy between photosystems to optimize linear electron transport or to act as a photoprotective mechanism for PS II (Allen et al. 1985, Biggins et al. 1985, Mullineaux and Allen 1988, Mullineaux and Holzwarth 1991, Mullineaux et al. 1991, Mullineaux 1992, Bruce and Salehian 1992, Salehian and Bruce 1992).

Although a model for the state transition consistent with all results remains elusive it is clear that any mechanism for the regulation of energy transfer efficiency between antenna pigments (PBS and/or Chl *a*) and reaction centres must necessarily invoke changes in chromophore separation and/or orientation.

Our results confirm that chromophore orientation changes accompany the state transition in cyanobacteria. We have shown that state 2 is characterized by increased alignment of the transition dipole of an APC core component of the PBS and decreased alignment of the Q_y transition of a Chl *a* component of PS II with both the long axis of the intact cell and the thylakoid membrane plane. The changes in orientation of APC and PS II associated Chl *a* could reflect conformational changes of the PBS and PS II complex themselves or changes in the association of these protein complexes with each other and/or other protein constituents of the thylakoid membrane.

Specific changes in APC orientation with the state transition were observed in an earlier room temperature LD study of intact cells but were not resolvable in PBS-thylakoid membrane fragments isolated from these cells (Brimble and Bruce 1989). These earlier results suggested that the changes in APC dichroism were dependent on the integrity of the intact cell, and were thus due to changes in the macroscopic order of PBS with respect to the intact cell. However, the increased resolution of the 77 K LD/A spectra of this report

has clearly shown increased alignment of the transition dipole of an APC core component in PBS-thylakoid membrane fragments isolated from cells in state 2. This result is consistent with a local change in orientation of an APC component with respect to the thylakoid membrane plane, rather than a change in macroscopic order.

The APC core of the *Synechococcus* hemidiscoidal PBS is composed of two cylinders laying side by side on the thylakoid membrane. Each cylinder is formed from four individual trimer-like discs, each containing α and β subunits of APC, see MacColl and Guard-Friar for review (1987). The α and β subunits each contain a single phycocyanobilin chromophore covalently bound to a cysteine residue. The absorbance maxima of isolated disks vary from 650 nm to 670 nm. They include the long wavelength fluorescing apc B and apc E gene products which have absorbance maxima at 670 nm and 655 nm respectively (Bryant 1991). These long wavelength APC components are considered to be the bridges for excitation energy from the PBS to PS II.

Although it is tempting to suggest that movement of one or both of these red-shifted APC components is responsible for the state transition induced change in dichroism observed in our study, strong overlap between the absorption bands of all APC components makes positive identification impossible. Our results could be explained by a change in orientation of one or more individual APC components, the entire core or the entire PBS. Any of these possibilities would be prime candidates for a molecular model of the state transition in cyanobacteria in which excitation energy transfer from PBS to PS II is affected.

Two such models for the state transition have been proposed, the mobile antenna model (Allen et al. 1985) and the PBS detachment model (Mullineaux and Allen 1988). However the simple mobile antenna model is confounded by evidence which indicates that Chl *a* is heavily involved in the mechanism (see review by Dominy and Williams 1987) and the PBS-detachment model is inconsistent with photoacoustic measurements indicating that there is no change in the efficiency of energy transfer from the PBS to the thylakoid membrane (Mullineaux et al. 1991, Bruce and Salehian 1992).

The small change in PS II associated Chl *a* orientation we observed in this study suggests direct involvement of PS II in the mechanism of the state transition. This is not inconsistent with any of the models for the state transition as all propose involvement of PS II either by a change in energy transfer from PBS to PS II (both mobile antenna and PBS detachment models) and/or from PS II to PS I (both spillover and PBS detachment models).

The relative change in PS II associated Chl *a* orientation with the state transition is much smaller than that observed for APC. This result cannot be used to quantify relative movements of PS II and PBS protein complexes as not all orientation changes of these complexes would necessarily be detectable by linear dichroism. However, the large change in APC dichroism observed in this study does suggest an important role for the PBS in the mechanism of the state transition. This concept is supported by evidence from room temperature and low temperature fluorescence yield changes with the state transition which suggest more involvement of PBS than Chl *a* in the mechanism (Mullineaux and Allen 1988, Mullineaux and Holzwarth 1991, Salehian and Bruce 1992). In apparent conflict with these results a PBS-less mutant of the cyanobacterium *Agmenellum quadruplicatum* has been shown to undergo state transitions as assayed by 77 K fluorescence emission (Bruce et al. 1989). That study was interpreted as evidence that the state transition in cyanobacteria did not require PBS, and most likely involved spillover from PS II Chl *a* to PS I Chl *a*.

It is impossible to resolve these and other apparent conflicts in the literature without introducing some additional degree of freedom in the proposed mechanisms for the state transition in cyanobacteria. The mechanism is clearly complex and appears to involve independent changes in the distribution of PBS and Chl *a* absorbed energy to PS II and PS I and/or independent changes in excitation energy distribution and Chl *a* fluorescence yields. Our current results indicate, however, that changes in the orientation of APC and

PS II associated Chl *a* are key components of the mechanism.

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