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Pigment orientation and excitation energy transfer in *Porphyridium cruentum* and *Synechococcus* sp. PCC 6301 cross-linked in light state 1 and light state 2 with glutaraldehyde

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Phycobilisome/thylakoid membrane fragments were isolated from the red alga *Porphyridium cruentum* and the cyanobacterium *Synechococcus* sp. PCC 6301. Intact cells were cross-linked with glutaraldehyde in light state 1 or light state 2, broken with a French press and membrane fractions isolated by sucrose density gradient centrifugation. For both species a fraction was isolated which contained phycobilisomes energetically coupled to the thylakoid membrane. The 77 K fluorescence emission spectra of these phycobilisome/thylakoid membrane fragments retained the relative PS II and PS I Chl *a* fluorescence emission yields characteristic of the light state in which the cells had originally been cross-linked. A comparison of linear dichroism spectra of the isolated phycobilisome/membrane fragments with intact cells showed that the significant change in allophycocyanin orientation previously reported to accompany the state transition in the cyanobacteria *Synechococcus* sp. PCC 6301 (Bruce, D. and Biggins, J. (1985) Biochim. Biophys. Acta 810, 295–301) was not observed in the isolated membrane fragments. Linear-dichroism spectra of the membrane fragments from *P. cruentum* in state 1 and state 2 showed a small change in phycoerythrin orientation but no change in allophycocyanin orientation. As observation of the change in allophycocyanin orientation was dependent on the integrity of the intact cell, we conclude that the change reflects an orientation with respect to the long axis of the cell and not an orientation with respect to the thylakoid-membrane plane.

Introduction

Both LHC Chl *a/b*- and PBS-containing photosynthetic organisms regulate the distribution of excitation energy between PS II and PS I by a mechanism known as the light state transition [1,2]. The photosynthetic apparatus responds to a preferential excitation of one photosystem by changing the distribution of excitation energy in favour of the other photosystem.

In LHC Chl-*a/b*-containing organisms the molecular basis for this regulatory response has been determined to involve the reversible phosphorylation of a mobile LHC2 Chl *a/b* complex [3]. The phosphorylation is triggered by reduction of intersystem electron carriers [4]. Upon phosphorylation, a lateral migration of the LHC2 Chl *a/b* complex from the appressed thylakoid regions rich in PS II to the stroma membrane regions, rich in PS I, has been proposed to occur [5]. Regulation is therefore effected by a change in the relative association of the LHC2 Chl *a/b* complex with PS II and PS I. For a review of the state transition in Chl-*a/b*-containing organisms, see Refs. 6 and 7.

Cyanobacteria and red algae do not contain a membrane-bound LHC Chl complex, but instead have water-soluble phycobiliproteins as accessory antenna pigments. The phycobiliproteins are organized into peripheral membrane-associated complexes, known as PBS [8], which are associated predominantly with PS II [9]. These large complexes presumably prevent appression of the thylakoid membranes and there is no evidence to suggest heterogeneity in the distribution of PS II and PS I in the thylakoid membrane.

The kinetics and energetic requirements of the light state transition in PBS-containing organisms have been shown to differ from those described for Chl-*a/b*-con-

Abbreviations: Chl *a*, chlorophyll *a*; PBS, phycobilisome; PS, Photosystem; LHC, light-harvesting complex; APC, allophycocyanin.

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taining organisms [10–12]. A working model for the state transition in PBS-containing organisms has been proposed, suggesting that regulation results from control of the separation between the two photosystems affecting the rate of energy transfer from PS II Chl *a* to PS I Chl *a* [13]. This proposed mechanism did not involve a mobile antenna complex or protein phosphorylation.

More recently, a mechanism analogous to that of Chl-*a*/*b*-containing organisms has been proposed for PBS-containing organisms. In this model, the PBS is a mobile antenna and associates with PS I upon phosphorylation of PBS and PS II components (state 2) and with PS II upon dephosphorylation of these components (state 1) [14]. There is, however, no evidence from excitation-energy-transfer studies for a direct coupling of the PBS with PS I in state 2 and the requirement of phosphorylation in the state transition mechanism has been challenged [15]. For a review of state transitions in PBS-containing organisms, see Refs. 16 and 17.

Efforts toward elucidating the detailed mechanism of the state transition in PBS-containing organisms have been frustrated by difficulties in obtaining a suitable in vitro PBS/thylakoid membrane preparation. PBS/thylakoid membrane and PBS/PS II preparations have been isolated which show high rates of electron transport [18,19] but are not competent to effect state transitions (Bruce and Biggins, unpublished data). To maintain the association of the PBS with the thylakoid membrane in these preparations very high-molarity suspension buffers are required (i.e., 0.5 M phosphate/0.26 M citrate/0.5 M sucrose [19]), which may inhibit the state transition.

It is possible to maintain the integrity of the PBS and of the PBS-thylakoid association by the use of protein cross-linking agents. The technique of protein cross-linking with glutaraldehyde has been used to stabilize isolated intact PBS and PBS/thylakoid membrane preparations [20] and PBS/thylakoid membrane fragments have been isolated after glutaraldehyde treatment of intact cells [21]. Cross-linking with glutaraldehyde has also been used to chemically stabilize intact cells of red algae and cyanobacteria in state 1 and state 2 [22].

In vivo studies have determined the pathway of excitation energy transfer for cells in state 1 and state 2. Measurements of 77 K fluorescence induction kinetics [23] and picosecond fluorescence kinetics [24,25] indicate that the regulation is effected by a change in the rate constant for excitation energy transfer from PS II Chl *a* to PS I Chl *a*. This change in the efficiency of energy transfer could be caused by changes in pigment separation or orientation.

There is evidence for a pigment-orientation change correlated with the state transition in the cyanobacteria *Synechococcus* sp. PCC 6301 [26]. A difference in the linear-dichroism spectra of intact cells chemically cross-linked in state 1 and state 2 indicated a change in the orientation of APC, a PBS core component absorbing maximally at 656 nm. Electron microscopy has also revealed changes in the organization of the PS2-containing EF particles in the thylakoid membrane correlated with the state transition in cyanobacteria [27].

The objective of this study was two-fold: first, to isolate a PBS/thylakoid membrane fraction in state 1 or state 2 and, second, to investigate possible pigment-orientation changes accompanying the state transition in both the cyanobacteria *Synechococcus* sp. PCC 6301 and the red algae *P. cruentum*. PBS/thylakoid membrane fragments isolated from cross-linked cells of both species exhibited 77 K fluorescence emission spectra characteristic of the light state into which the intact cells had originally been cross-linked. Although intact cells of *Synechococcus* sp. PCC 6301 did show the orientation change of APC previously correlated with the state transition [26], the membrane fragments from both species showed no changes in APC orientation. We conclude that the change in linear dichroism which accompanies the light-state transition in *Synechococcus* sp. PCC 6301 is due to a change in the orientation of APC with respect to the long axis of the intact cell and not to the plane of the thylakoid membrane.

Materials and Methods

P. cruentum was grown autotrophically at a light intensity of 25 $\mu E \cdot m^{-1} \cdot s^{-1}$ on artificial seawater medium [28]. *Synechococcus* sp. PCC 6301 was grown autotrophically at a light intensity of 25 $\mu E\ m^{-2} \cdot s^{-1}$ on BG-11 [29]. Cells were harvested in late exponential phase and resuspended in their growth media to an absorbance of 1.0 at 680 nm. In both species, state 1 was induced by illumination with blue light (Ditric optics 460 nm short-pass filter) for 4 min at 350 $\mu E \cdot m^{-2} \cdot s^{-1}$. State 2 was induced in *P. cruentum* with green light (Ditric optics 520 nm long pass and 600 nm short-pass filters) or with orange light (Ditric optics 580 nm long pass and 600 nm short-pass filters) in *Synechococcus* sp. PCC 6301 for 4 min at 350 $\mu E \cdot m^{-2} \cdot s^{-1}$. Cells were then chemically cross-linked by the addition of glutaraldehyde to a 1.2% final concentration in *P. cruentum* and a 0.5% final concentration in *Synechococcus* sp. PCC 6301 as described previously [22]. Cells cross-linked in either state 1 or state 2 were passed twice through an Aminco French pressure cell (16000 p.s.i.) and centrifuged for 10 min at $1000 \times g$ to remove unbroken cells and any large broken cell material. The supernatant was loaded onto a 20 ml continuous-sucrose gradient (0.2–1.75 M). The gradient was floated on a 4 ml 2 M sucrose cushion. All sucrose solutions contained 20 mM tricine (pH 8.0). The gradients were spun for 6 h at $50000 \times g$. In experiments with *P. cruentum*, a red band was removed from the 1.75 M/2 M interface, and

in those with *Synechococcus* sp. PCC 6301, a blue-green band was removed at approx. 0.5 M sucrose. In both species, the isolated fractions contained PBS and Chl *a* as determined by absorbance spectroscopy. Typically, 70–80% of the pigment loaded onto the gradient was recovered in these fractions. Low-temperature fluorescence emission indicated that the PBS were energetically coupled to the membranes.

Absorption spectra were recorded on an Aminco DW 2 spectrophotometer with frosted glass to correct for light-scattering distortion. The DW-2 was interfaced to an IBM-compatible computer by OLIS (Georgia). Fluorescence-emission spectra were obtained with a spectrofluorimeter based on a Jarrel Ash 0.25 meter spectrograph and EG&G diode array detector (1420R) controlled by an EG&G detector interface (1461) accessed by an IBM-AT-compatible computer. Excitation light (10 nm bandwidth) for the fluorescence emission was supplied by a 100 W tungsten halogen lamp, dispersed by a Jobin Yvon H20 spectrometer.

Linear dichroism was measured with a home-built spectrophotometer using a Hinds photoelastic modulator and EG&G 5207 lock-in amplifier. Light from a 50 W tungsten halogen lamp was dispersed through a Jobin Yvon H20 spectrometer and directed through a calcite Glans Thompson polarizer, before passing through the modulator and sample holder toward a Hammamatsu R562HA end-on photomultiplier tube in a mu metal shield. Both spectrometer control and data collection were performed by an IBM-XT-compatible computer via an interface and pulse-encoded motor controller constructed by the Brock University electronics shop.

Intact cells and membranes were oriented for linear-dichroism measurements using the squeezed-gel technique [30]. The gel consisted of 10% acrylamide/1.5 M sucrose/20 mM tricine (pH 8.0).

The technique used for the gel orientation was similar to that described previously [26]. The sample cuvette was of a slightly different design and is described below. The gel-containing sample was cast into a U-shaped brass cuvette with optical glass windows in both legs. Two rubber dams sealed the open ends of the cuvette during the casting procedure. The gel completely filled the depth of the cuvette. After the gel had set, the rubber dams were removed and a cuvette holder was inserted to 1/3 of the depth of the cuvette. The compression of the gel by the cuvette holder forced it to expand along the long axis of the cuvette, perpendicular to the compression axis. The optical path through the cuvette windows was perpendicular to both the compression axis and the expansion axis. The linear dichroism reported is the difference in absorption of light polarized parallel to the gel-expansion axis minus that of the light polarized perpendicular to the gel-expansion axis.

Results

Fig. 1 compares the 77 K fluorescence-emission spectra of intact cells (panel A) of *Synechococcus* sp. PCC 6301 after fixation into state 1 and state 2 with the spectra of PBS/membrane fragments (panel B) isolated from these cells. The actinic wavelength (590 nm) was chosen to excite phycocyanin. The fluorescence-emission spectra for cross-linked cells in state 1 and state 2 were very similar to those reported previously for intact cells [26,27] and showed the typical increase in PS II Chl *a* (685, 695 nm) emission yield relative to PS I Chl *a* (720 nm) emission in state 1. The PBS/membrane fragments retained the relative Chl *a* fluorescence emission characteristic of the light state of the cross-linked cells they were isolated from. As compared to the cross-linked cells, the membrane fragments showed an increased phycobiliprotein fluorescence yield and a relatively lower 695 nm peak with respect to the 685 and 720 nm peaks.

In Fig. 2, the 77 K emission spectra of intact cells of *P. cruentum* cross-linked in state 1 and state 2 (panel A) are compared to the emission spectra of PBS/thylakoid membrane fragments isolated from these cells (panel B). The actinic wavelength (560 nm) was chosen to excite phycoerythrin. As observed for *Synechococcus* sp. PCC 6301 in Fig. 1, the fluorescence-yield changes indicative of the light-state transition were observed in both the intact cross-linked cells and the membrane fragments isolated from them. As also seen in *Synechococcus* sp. PCC 6301, the fluorescence yield of the phycobiliprotein was increased relative to the Chl *a* and the yield of the 695 nm peak was decreased relative to the 685 and 720 nm peaks.

Fig. 3 compares the 77 K fluorescence-emission spectra of the samples shown in Fig. 2 with the actinic light

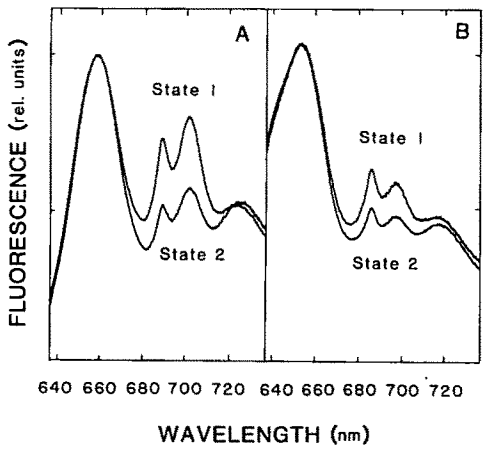

Fig. 1. 77 K fluorescence-emission spectra of intact cells of *Synechococcus* sp. PCC 6301 cross-linked in state 1 or state 2 with glutaraldehyde (A) and PBS/thylakoid membrane fragments (B) isolated from these cells as described in Materials and Methods. The excitation wavelength was 590 nm.

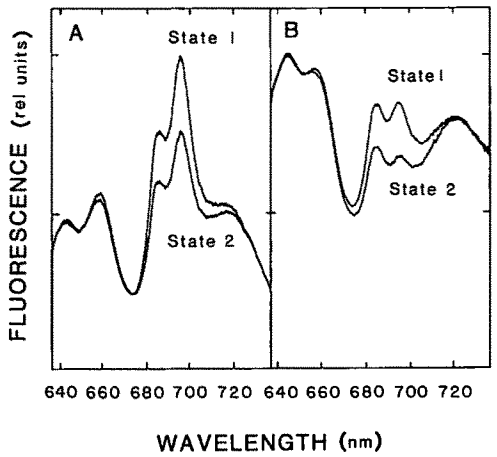

Fig. 2. 77 K fluorescence-emission spectra of intact cells of *P. cruentum* cross-linked in state 1 or state 2 with glutaraldehyde (A) and PBS/thylakoid membrane fragments (B) isolated from these cells as described in Materials and Methods. The excitation wavelength was 560 nm.

chosen to excite Chl *a* (435 nm). Again, fluorescence-emission characteristic of cells in state 1 and state 2 was observed in both cross-linked cells (A) and PBS/thylakoid membrane fragments (B). The difference between state 1 and state 2 appears small due to the dominance of the PS I Chl *a* emission at 720 nm. The PBS/thylakoid membrane fragments had a higher yield of 685 nm emission relative to 720 nm emission than the cross-linked cells.

The differences, state 1 minus state 2, in the fluorescence-emission spectra in Figs. 2 and 3 are shown in Fig. 4. The amplitude of the difference spectra of both cross-linked cells and membrane fragments were much lower for excitation at 435 nm (traces C and D) than for excitation at 560 nm (traces A and B). However, when the contribution of the PS I emission (720 nm peak) was

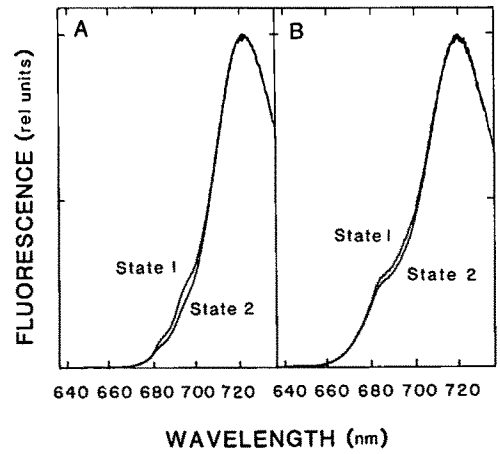

Fig. 3. 77 K fluorescence-emission spectra of intact cells of *P. cruentum* cross-linked in state 1 or state 2 with glutaraldehyde (A) and PBS/thylakoid membrane fragments (B) isolated from these cells as described in Materials and Methods. The excitation wavelength was 435 nm.

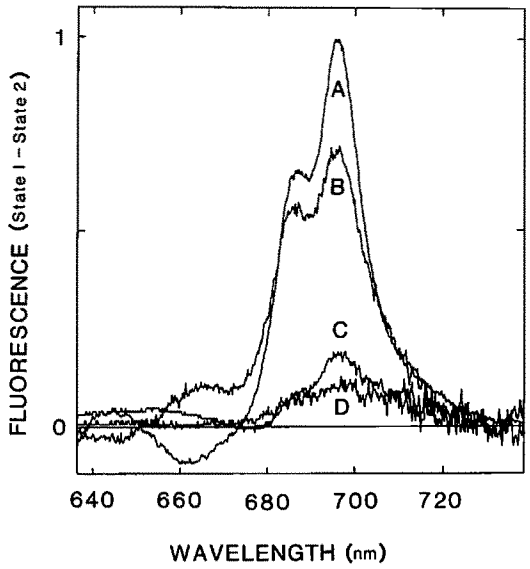

Fig. 4. Difference spectra, state 1 minus state 2, of the fluorescence-emission spectra shown in Figs. 2 and 3. (A) glutaraldehyde cross-linked cells (excitation at 560 nm); (B) PBS/thylakoid membrane fragments (excitation at 560 nm); (C) glutaraldehyde cross-linked cells (excitation at 435 nm); (D) PBS/thylakoid membrane fragments (excitation at 435 nm).

subtracted before the spectra were normalized, the difference spectra were similar in amplitude (not shown).

All difference spectra peaked at 695 nm. For excitation at both 560 nm (traces A and B) and 435 nm (traces C and D) the amplitude of the difference spectra was higher in the cross-linked cells (traces A and C) than in the isolated membrane fragments (traces B and D). Minor changes were also observed in the phycobilin region at 660 nm. We found these changes to be variable, but it has been reported that the yield of phycocyanin (660 nm) increases slightly in state 1 [31].

In Fig. 5 the absorbance spectrum of intact cells of *Synechococcus* 6301 (dotted line) is compared to the spectra of PBS/thylakoid membrane fragments isolated

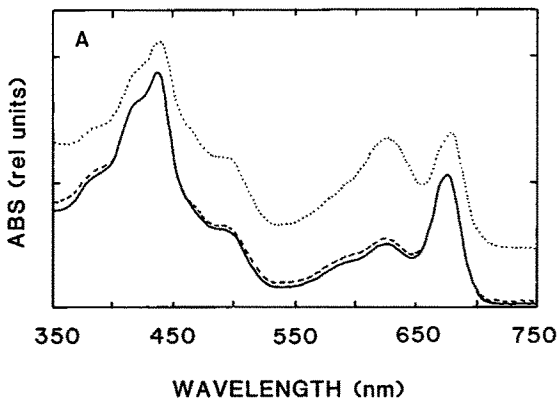

Fig. 5. Absorbance spectra (panel A) of intact cells of *Synechococcus* sp. PCC 6301, dotted line, and of the PBS/thylakoid membrane preparations isolated from cells cross-linked into state 1 (solid line) or state 2 (dashed line) with glutaraldehyde.

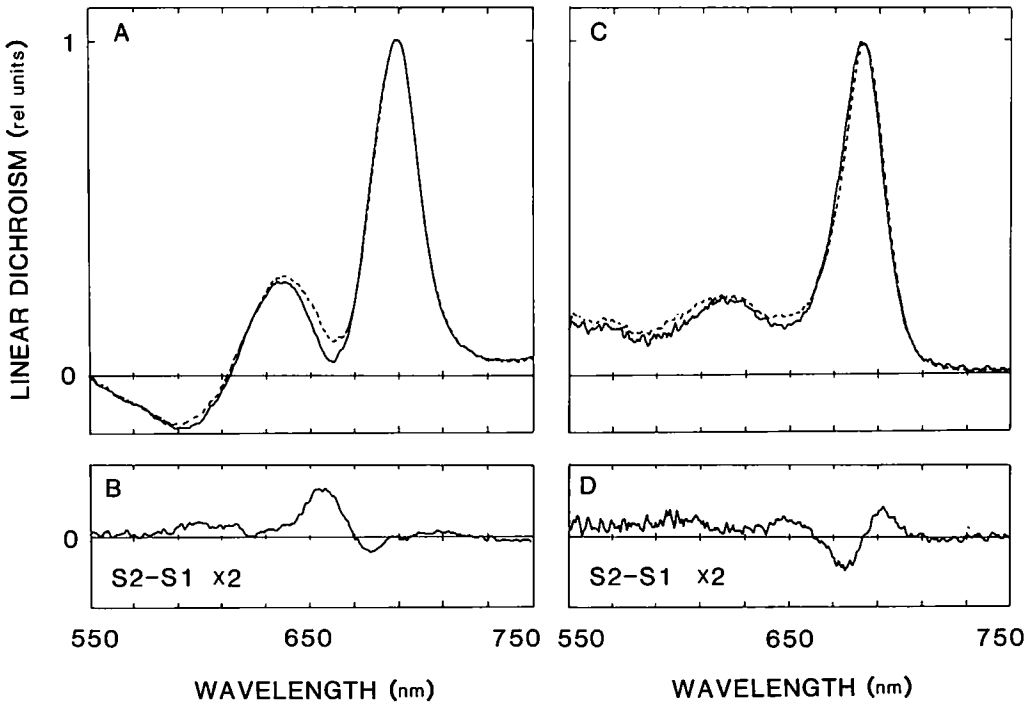

Fig. 6. Linear-dichroism spectra ($A_{\parallel} - A_{\perp}$) of intact cells of *Synechococcus* sp. PCC 6301 cross-linked in state 1 and state 2 (panel A) and of the PBS/thylakoid membrane fragments isolated from these cells (panel C). The difference spectra, state 2 minus state 1, for intact cells are shown in panel B and for PBS/thylakoid particles in panel D. The difference spectra have been multiplied by 2.

from cells cross-linked in state 1 (solid line) and state 2 (dashed line). The absorbance spectra for membrane fragments in state 1 and state 2 were very similar. The absorbance spectra of the membrane fragments were similar to the spectrum for intact cells, but showed a decrease in the amount of phycobilin relative to Chl *a*.

The room-temperature linear-dichroism spectra of intact cells of *Synechococcus* sp. PCC 6301 cross-linked in state 1 and state 2 (panel A) are compared to the membrane fragments isolated from these cells (panel C) in Fig. 6. As shown previously at 77 K [26], the difference spectrum, state 2 minus state 1 (panel B), has a major peak at 656 nm. A small negative peak was observed at 677 nm in the difference spectrum and reflects a small reproducible red shift (0.5 nm) of the Chl *a* peak (688 nm) on transition to state 2. The spectra were complicated in the phycobilin region with a positive peak at 637 nm and a negative peak at 592 nm. The linear-dichroism spectra of the membrane fragments were considerably simpler than those of the intact cells. The membrane fragments showed a lower contribution by the phycobilin pigments and were very similar for state 1 and state 2. The short wavelength peak was at 622 nm and the Chl *a* peak at 683 nm (a 5 nm blue shift with respect to the intact cells). The linear-dichroism spectra of the membrane fragments showed less light-scattering distortion as evidenced by the amplitude of the signal from 720 to 750 nm. The difference spectrum (panel D) of state 2 minus state 1 membrane fragments showed a small general increase in dichroism from 550 to 650 nm in state 2 relative to the Chl *a* peak, but there was no specific change at 656 nm as was observed in the intact cells. The difference

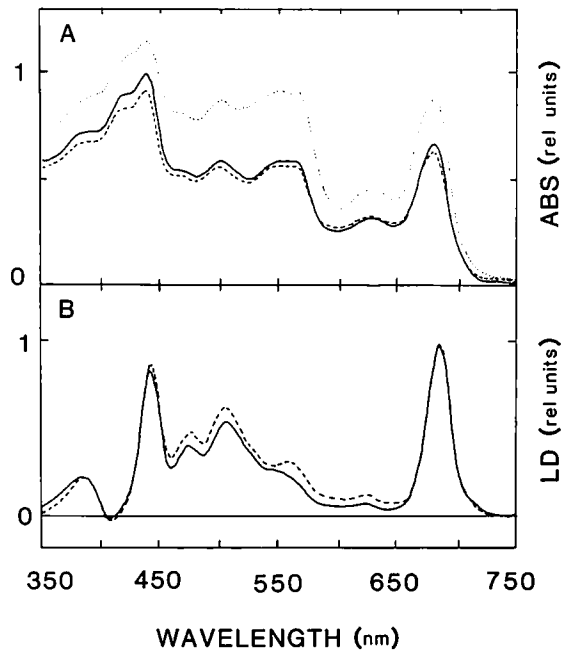

Fig. 7. Absorbance spectra (panel A) of intact cells of *P. cruentum* (dotted line) and of the PBS/thylakoid membrane preparations isolated from cells cross-linked into state 1 (solid line) or state 2 (dashed line) with glutaraldehyde. Linear-dichroism spectra (panel B) of PBS/thylakoid membrane preparations isolated from cells cross-linked into state 1 (solid line) or state 2 (dashed line) with glutaraldehyde.

spectrum did show a negative peak at 675 nm and a positive peak at 692 nm, which resulted from a red shift of the Chl *a* peak in state 2. This red shift (approx. 1 nm) is more pronounced than that observed in the intact cells.

The absorbance spectra (panel A) of intact cells and PBS/thylakoid membrane fragments from *P. cruentum* are compared to the linear-dichroism spectra (panel B) of the membrane fragments in Fig. 7. The PBS/thylakoid membrane fragments had absorbance spectra similar to the intact cells (dotted line) and there was no significant difference between the absorbance spectra for membrane fragments in state 1 (solid line) and state 2 (dashed line). The PBS/thylakoid membrane fragments had lower phycobilin absorption than the intact cells indicating some loss of phycoerythrin and phycocyanin.

The linear-dichroism spectra for the PBS/thylakoid membrane fragments shown in Fig. 7 panel B exhibit positive dichroism for the Chl *a* Q_y transition (682 nm) and the Chl *a* Soret band (442 nm). The spectra also show positive peaks in the carotenoid region (470 nm and 500 nm) and the phycoerythrin region. The linear-dichroism spectra were similar for PBS/thylakoid membrane fragments in state 1 (solid line) and state 2 (dashed line). There was, however, a small general increase in dichroism from 450 to 650 nm in state 2 and a difference in the position of the phycoerythrin peak between membrane fragments in state 1 (553 nm) and state 2 (560 nm). Although not readily discernable in Fig. 7, expanded plots comparing the state 1 and state 2 linear-dichroism spectra showed a small red shift (0.5 nm) of both the Chl *a* Q_y peak at 682 nm and the Soret peak at 440 nm (data not shown).

Discussion

PBS / thylakoid membrane fragments

In confirmation of an earlier study [21], we show that PBS/thylakoid membrane fragments can be isolated from cells of *P. cruentum* which have been cross-linked with glutaraldehyde. The PBS do not dissociate and they remain energetically coupled to Chl *a* in the thylakoid membrane. We also show that this technique can be applied to the cyanobacterium *Synechococcus* sp. PCC 6301.

As compared to intact cells of both species, the PBS/thylakoid membrane fragments showed an increase in phycobilin fluorescence relative to Chl *a* emission, even though the absorbance spectra of the membrane fragment preparations showed some loss of phycobilins. The increased phycobilin fluorescence therefore indicated either some uncoupling of energy transfer through the phycobilin pigments or a contamination of free phycobiliproteins in the preparation. A small contamination of uncoupled phycobiliproteins would not add significantly to the absorbance spectra but would, due to their high fluorescence yield, greatly affect the fluorescence emission spectra.

The high yield of Chl *a* emission resulting from excitation of either phycoerythrin or phycocyanin does, however, indicate that much of the PBS remains functionally associated with the Chl *a* of the thylakoid membrane in the membrane-fragment preparations of both species. Moreover, the much higher yield of PS II Chl *a* fluorescence relative to PS I Chl *a* emission observed with excitation of the PBS, as compared to excitation of Chl *a*, indicated the preferential association of the PBS with PS II in the membrane fragments and in the intact cells of both species.

Fluorescence-yield changes

The PBS/thylakoid membrane fragments isolated from cells cross-linked into state 1 or state 2 retained the 77 K fluorescence emission spectra characteristic of each light state. As shown previously with intact cells [23,31], the 77 K fluorescence-emission spectra of both cross-linked cells and PBS/thylakoid membrane fragments in state 1 and state 2 show the fluorescence yield changes characteristic of the state transition when actinic wavelengths are chosen to excite either the PBS (560 nm) or Chl *a* (435 nm). The state 1 minus state 2 difference spectra of cross-linked cells and PBS/thylakoid membrane fragments peak at 695 nm, regardless of excitation wavelength, as the difference spectra of intact cells have been shown to do previously [23,31]. A mechanism for the state transition based on the regulation of excitation energy transfer from PS II Chl *a* to PS I Chl *a* predicts this result in contrast to the mobile PBS mechanism [14], which predicts that the fluorescence emission changes indicative of the state transition should be detected only with excitation absorbed by the PBS. The fluorescence emission and difference spectra indicate that the central role of PS II Chl *a* (695 nm) in the mechanism of the state transition has been maintained in the isolated PBS/thylakoid membrane fragments.

Pigment orientation in P. cruentum

The linear-dichroism spectra of the PBS/thylakoid membrane fragments isolated from *P. cruentum* show a higher orientation of the Chl *a* Q_y and Soret transitions relative to the phycobilin transitions. The spectra did not show much contribution from APC or phycocyanin, although the phycoerythrin peak was pronounced. A previous linear-dichroism study of isolated thylakoid membranes with PBS attached [20] from *Porphyridium* sp. Lewin showed similar contributions. However, in that study, the relative contributions in the APC and phycocyanin region were much higher. They proposed the orientation of the APC transition at 655 nm to be closer to the membrane plane than the 680 nm Chl *a*

transition. Our data suggest that phycocyanin and APC are not highly oriented in the PBS/thylakoid membrane fragments. The differences between the two studies may result from the different techniques used to isolate the PBS/thylakoid membrane fraction. In the previous study, thylakoid membranes with PBS attached were isolated in 0.75 M phosphate buffer. To maintain the PBS/thylakoid association the isolated membranes were cross-linked with glutaraldehyde and the phosphate removed to facilitate electric linear-dichroism measurements. In this study the intact cell was cross-linked and PBS/thylakoid membrane fragments isolated afterwards. It is more likely that the technique used in this study maintains the in vivo conformation of the PBS and thylakoid membrane, as the PBS is cross-linked to the membrane before isolation.

There were two small reproducible differences between the linear-dichroism spectra of membrane fragments from *P. cruentum* in state 1 and state 2. The phycoerythrin peak was observed to shift from 553 nm in state 1 to 560 nm in state 2 and there was a small red shift in the Chl *a* Q_y peak in state 2. There were no accompanying changes in the absorbance spectra of these membrane fragments. The observed change in phycoerythrin orientation is not correlated with a change in the efficiency of excitation energy transfer from phycoerythrin to phycocyanin. No change has been reported for phycoerythrin fluorescence yield or lifetime with the state transition in *P. cruentum* [23–25]. The small red shift of the Chl *a* peak may indicate an orientation change of the Chl *a* with respect to the thylakoid membrane plane or a difference in the light-scattering properties of the PBS/thylakoid membrane fragments in state 1 and state 2.

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

We confirm the change in dichroism previously reported [26] in the 77 K linear-dichroism spectra of intact cells of *Synechococcus* sp. PCC 6301 cross-linked in state 1 and state 2. We also observed a small red shift (0.5 nm) of the Chl *a* Q_y transition in state 2 which was not reported in the previous study [26]. This red shift was observed repeatedly and its detection was only possible due to the accuracy (0.1 nm) of the computer-controlled spectrometer drive used in the present study.

The change in dichroism at 656 nm was lost upon fractionation of the intact cells and isolation of the membrane fractions, although the red shift of the Chl *a* Q_y peak observed on transition to state 2 was retained. As the red shift of the Chl *a* peak in the membrane-fragment spectra was more pronounced than that found in the intact cells, it can not be due to changes in light scattering induced by changes in intact cell morphology. The band shift may therefore reflect an actual change in orientation of Chl *a* with respect to the thylakoid membrane plane, or a change in light scattering indicative of a structural difference in the PBS/thylakoid membrane fragments.

An analysis of the linear-dichroism results and comparison to ultrastructural studies

As discussed previously [26], the linear dichroism of intact cells of rod-shaped cyanobacteria like *Synechococcus* sp. PCC 6301 is potentially sensitive to two general types of orientation due to the cells regular concentric thylakoid membrane organization. Orientation with respect to the long axis of the cell and with respect to the plane of the thylakoid membrane can both contribute to the dichroism of the intact cell.

The standard analysis of transition dipole orientation applied to an oriented planar membrane system assumes a random orientation of the dipole about a normal to the membrane surface. Only the angle of the dipole with respect to the membrane plane can be determined. When the dipole is aligned parallel to the membrane plane, the result is a positive linear-dichroism signal, when perpendicular, a negative signal. However, an additional degree of order may exist in the system. For example, the concentric thylakoid membranes of *Synechococcus* sp. PCC 6301 are oriented along the long axis of the cell and this orientation is maintained during the measurement of intact cell linear dichroism.

Fig. 8a shows transition dipoles which are parallel to the membrane surface but oriented randomly about a normal to the membrane surface, as assumed for the planar membrane analysis. Under these conditions, analysis of the linear dichroism is very similar to the case for planar membranes and only determinations of the angle the dipole makes with the plane of the membrane surface are possible. It is apparent that the di-

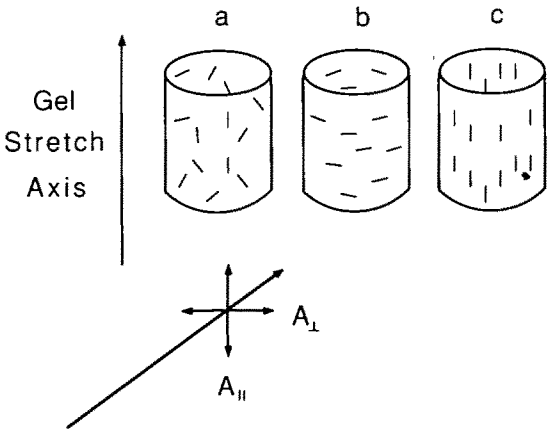

Fig. 8. Model showing the proposed in situ orientation of the cylindrical thylakoids of *Synechococcus* sp. PCC 6301 when oriented by the squeezed-gel technique for determination of linear dichroism. Transition dipoles parallel to the membrane plane are shown arranged (a) randomly about a normal to the membrane surface, (b) perpendicular to the long axis of the cell and (c) parallel with the long axis of the cell.

chroic ratio of this transition in the cylindrical membrane will be much lower than if it were in a planar membrane as so much less of the membrane (only the edges of the cylinder) is actually oriented parallel to the measurement axis. The situation is very different if the transitions are oriented with respect to the long axis of the intact cell, as shown in Fig. 8b and c. These transitions are all oriented parallel to the membrane plane as they were in Fig. 8a, but their additional orientation with respect to the long axis of the cylinder would cause 8b to show a large negative dichroism and 8c a large positive dichroism. It is also apparent that the linear-dichroism signal would be extremely sensitive to this kind of orientation.

Orientation with respect to the long axis of the intact cell is presumed to be lost upon disruption of the cell and isolation of PBS/thylakoid membrane fragments. Thus, only orientation with respect to the thylakoid membrane plane would contribute to the linear-dichroism spectra of isolated membrane fragments, while the linear-dichroism spectra of intact cells would be sensitive to both.

We have shown that the change in APC orientation correlated with the state transition in intact cells of *Synechococcus* sp. PCC 6301 is dependent on the integrity of the cell. The change in dichroism therefore reflects a change in orientation of APC with respect to the long axis of the intact cell and not to the plane of the thylakoid membrane. The change observed in the linear-dichroism spectra could be interpreted as either an increase in positive APC dichroism in state 2 (as shown in Fig. 8c), and/or as an increase in negative APC dichroism in state 1 (as shown in Fig. 8b). It is difficult to distinguish which change is occurring due to the interference of the positively dichroic peaks at 637 and 688 nm in the linear-dichroism spectra (Fig. 6). However, comparison of the spectra at room temperature (Fig. 6) with those at 77 K (Fig. 2 of Ref. 26) indicates that the narrowing of the two bordering peaks at low temperature causes a significant decrease in the dichroism at 656 nm which actually appears negative in state 1 at 77 K. This supports the idea that the change is indicative of an increase in the orientation of APC perpendicular to the long axis of the intact cell in state 1 as shown in Fig. 8b.

Further support for an increase in large-scale thylakoid membrane order in state 1 comes from ultrastructural studies. The organization of EF particles (which contain PS II) in *Synechococcus* sp PCC 6301 detected by freeze-fracture electron microscopy was reported to change from a relatively random pattern in state 2 to a more ordered pattern in state 1, where long rows of EF particles were apparent [27]. Changes in the pattern of PBS organization on the thylakoid membrane surface were proposed to accompany the changes in the EF distribution [27] as PBS frequently appear organized on the outer surface of the thylakoid with the same spacing as the EF particles [32]. The linear-dichroism results presented in this study are in agreement with the electron microscopy if the increased order of EF particles observed in state 1 is correlated with an increase in the alignment of the APC transition perpendicular to the long axis of the cell in state 1.

In summary, we report the isolation of PBS/thylakoid membrane fragments from cross-linked *P. cruentum* and *Synechococcus* sp. PCC 6301 which retain the fluorescence characteristics of the light state in which the intact cells were originally cross-linked. The orientation change of APC previously reported to occur in *Synechococcus* sp. PCC 6301 was shown to depend on the integrity of the intact cell and was therefore proposed to indicate an orientation of APC with respect to the long axis of the intact cell and not the thylakoid membrane plane. A small red shift of the Chl *a* Q_y transition in the linear-dichroism spectra was observed in both species which may indicate either a reorientation of Chl *a* or a state-dependent change in the light-scattering properties of the PBS/thylakoid membrane fragments.

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