ELSEVIER

Biochimica et Biophysica Acta 1459 (2000) 139–147

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Excitation energy transfer from phycobilisomes to photosystems: a phenomenon associated with the temporal separation of photosynthesis and nitrogen fixation in a cyanobacterium, *Plectonema boryanum*[1]

Hari S. Misra *, Suresh K. Mahajan

Molecular Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India

Received 15 February 2000; received in revised form 4 April 2000; accepted 6 April 2000

Abstract

Plectonema boryanum shows temporal separation of photosynthesis and nitrogen fixation under diazotrophic conditions. Low temperature fluorescence studies have shown that in vivo the nitrogen fixing and photosynthesizing cells are adapted to 'state 2' and 'state 1', respectively. During nitrogen fixation phycobilisomes seem to transfer excitation energy to photosystem I whereas during oxygenic photosynthesis the energy is transferred to photosystem II. The state 2 adapted N-phase cells failed to undergo transition to state 1 while P-phase cells exhibited state 1 to state 2 transition. The nitrogen fixing cells showed a decreased level of *psb*C transcript, lack of CP47 in thylakoid membrane, and presence of the F685 peak but absence of the F695 peak in 77 K fluorescence spectra. These results suggest that the metabolic and molecular changes associated with nitrogen fixation may favor direct energy transfer from the phycobilisomes to photosystem I. This should help the organism to achieve low photosystem II and high photosystem I activity to set temporal separation of nitrogen fixation and photosynthesis for photoautotrophic growth under diazotrophic conditions.

Keywords: Photosynthesis; Energy transfer; Phycobilisome; Photosystem I; *Plectonema*

1. Introduction

Cyanobacteria contain phycobilisomes (PBS), the large complexes of phycobiliproteins bound to the surface of the thylakoid membrane [1]. In most cyanobacteria, including *Plectonema boryanum*, the different phycobiliproteins are phycocyanin, phycoerythrin and allophycocyanin which absorb light at 625, 565 and 650 nm respectively [2]. In general the order of excitation energy transfer within the PBS is from phycoerythrin to phycocyanin to allophycocyanin [3]. Allophycocyanin transfers photons to the long wavelength absorbing terminal emitters of phycobilisomes that finally transfer them to the chlorophyll (Chl) *a*

Abbreviations: Chl, chlorophyll; P700, primary electron donor of PSI; PAGE, polyacrylamide gel electrophoresis; PBS, phycobilisome; PET, photosynthetic electron transport; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; Q_A, primary quinone electron acceptor; Q_B, secondary quinone electron acceptor; SDS, sodium dodecyl sulfate

* Corresponding author. Fax: +91-22-550-5151; E-mail: mbad@magnum.barct1.ernet.in

[1] A part of this work was presented as an abstract (H.S. Misra, in: Proceedings of the XVIth International Congress of Biochemistry and Molecular Biology (IUBMB), Vol. II, p. 412, 19–22 September 1994, New Delhi, India.

0005-2728/00/$ – see front matter
PII: S0005-2728(00)00123-7

containing photosystems. The structure and functions of PBS in different cyanobacteria have been extensively studied (see [4] for review). However, the mechanisms of energy transfer from phycobilisomes to Chl*a* containing photosystems is poorly understood. Several earlier studies had shown that PBS were associated with photosystem (PS) II [5–8], suggesting that the contribution of PBS to the action spectra of the primary electron donor of PSI (P700) might be due to 'spillover' of excitation from PBS–PSII to PSI [9]. However, the demonstration of state transition in cyanobacteria by physiologically relevant environmental factors, such as change in light quality and intensity and CO_2 limitation, and in PSII-deficient mutants has provided evidence for transfer of excitation energy from PBS to PSI (see [10–12] for reviews). The latter studies have mostly used cells either adapted to monochromatic light or bearing mutations in the *psb*C and *psb*D genes [12]. This phenomenon has not been studied with cells growing photoautotrophically while performing PSII-independent and light requiring processes. Here we report such a study and show that the transition to state 2 may be promoted by an adaptive mechanism which permits the cells to carry out PSII-independent and light-dependent nitrogen fixation and anoxygenic photosynthesis in the cyanobacterium, *Plectonema boryanum* [13].

Plectonema, a microaerophilic nitrogen fixer [14], shows temporal separation of photosynthetic (P-phase) and nitrogen fixation (N-phase) activities while growing diazotrophically in continuous light. The N-phase cells show characteristic features such as a decrease in PSII activity, an increase in PSI activity, and a decrease in intracellular nitrogen reserve and PSII-independent CO_2 fixation [13]. Nitrogen fixation in this organism occurs when the plastoquinone (PQ) pool is reduced and electron transport between the primary and secondary quinone electron acceptors (Q_A and Q_B) is impaired [15] leading to uncoupling of the PSII and PSI activities [16]. These metabolic changes and their association with nitrogen fixation make *P. boryanum* an ideal system to study the excitation energy distribution from PBS to photosystems. Here we report that this organism uses a regulatory mechanism that is similar to state transition to separate nitrogen fixation and oxygenic photosynthesis to accomplish diazotrophic growth. Wild-type cells with different redox states of photosynthetic electron transport (PET) components showed differences in CP47 assembly in thylakoids, in the expression of the *psb*C gene and in transfer of excitation energy from the PBS to the photosystems. Nitrogen fixing cells with low expression of the *psb*C gene and decreased levels of CP47 in the thylakoid membrane showed altered excitation energy transfer from PBS to the two photosystems indicating that the metabolic changes associated with nitrogen fixation may alter the specificity of excitation energy transfer from PBS to photosynthetic reaction centers.

2. Materials and methods

2.1. Organisms and growth conditions

P. boryanum UTEX 594 is from the University of Texas Collection. Under nitrogen-sufficient conditions, it was grown in BG11 medium [17] bubbled with air passed through a 0.22 μm filter at 25 ± 2°C under continuous illumination as described [18]. Photoautotrophic growth under diazotrophic conditions was established with continuous bubbling of a mixture of N_2 and CO_2 as described [13].

2.2. Fluorescence spectroscopy

The cells were harvested by centrifugation and suspended in $BG11_0$ at a Chl*a* concentration of 5 μg/ml. They were incubated in the dark at 25°C for 30 min in a microaerobic atmosphere, then illuminated with white fluorescent light for 30 min and frozen in liquid nitrogen under constant illumination as described earlier [19]. The fluorescence emission spectra were recorded at 77 K on a Perkin- Elmer LS-5 spectrofluorimeter at excitation wavelengths 437 nm and 546 nm, respectively. Average values from triplicate spectra of each sample were used for plotting the emission spectra that were normalized at the phycocyanin (650 nm) and the PSII (685 nm) peaks.

2.3. Probe preparation and Southern hybridization

The preparation of plasmid DNAs and the elution of DNA fragments from agarose gel were done as

described [20]. For preparation of probes other than *psb*B (Table 1), the appropriate plasmid DNA was digested with appropriate restriction endonucleases and separated on 1% low melting point agarose. The correct size DNA fragments were excised and eluted from agarose. For the *psb*B gene probe, PCR-amplified full-length *psb*B gene DNA (1.529 kb) was digested with *Hin*dIII and a 623 bp fragment was eluted from the agarose gel. DNA probes were radiolabelled using [^{32}P]αdCTP (Board of Radiation and Isotopes Technology, Department of Atomic Energy, India) and the random primed DNA labeling kit (Roche Biochemicals, Germany) following the manufacturer's protocols. For Southern hybridization, the total DNA from *Plectonema* was prepared according to a modified protocol of Felkner and Barnum [21]. The cyanobacterial culture grown to mid-exponential phase in BG11 medium was harvested and washed in 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA. One gram of the cell pellet was suspended in 4 ml sucrose buffer (50 mM Tris–HCl pH 8.0, 100 mM EDTA, 25% w/v sucrose). Lysozyme was added to a final concentration of 5 mg/ml and incubated for 1 h at 37°C. The suspension was treated with proteinase K (100 μg/ml) and incubated at 37°C in the presence of 1% sodium dodecyl sulfate (SDS) until it become clear. The cleared lysate was extracted with phenol and chloroform. The subsequent steps were as described [18]. Total DNA (5.0 μg) was digested with appropriate restriction enzymes, treated for 30 min at 37°C with DNase-free RNase (50 μg/ml) and electrophoresed on 1% agarose. The DNA fragments were transferred to a nylon membrane. Prehybridization and hybridization were carried out as described by Sambrook et al. [20].

2.4. RNA isolation and Northern hybridization

Total RNA from N-phase and P-phase cells was isolated using a modified protocol of Chomczynski and Sacchi [22]. Cells were harvested at 4°C, the pellet was washed with 50 mM Tris–HCl (pH 8.0) containing 100 mM NaCl and immediately frozen at −70°C and then thawed. A high concentration of guanidinium thiocyanate (5 M) was used and the cells were broken by vortexing with acid-washed sterilized glass beads (0.45–0.60μm, Sigma Chemical Co., USA) as described [23]. Additionally, the RNA pellet was further washed with 3 M sodium acetate (pH 5.6–6.3) to reduce DNA contamination in the preparation. The RNA concentration was determined spectrophotometrically. For dot blot hybridization the RNA samples were denatured with formaldehyde–formamide and blotted on Hybond N^+ (Amersham Pharmacia Biotech, USA) using a manifold apparatus as described by Sambrook and colleagues [19]. The prehybridization and hybridization were carried out at high stringency as described earlier [24]. Detection of signals and quantitation of probes hybridized to specific RNA were achieved using a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA). The blot hybridized with *psb*D was boiled in 0.1% SDS for 5 min to strip off the probe and then reprobed with *psb*C and subsequently with the 23S rRNA (*rrn*) gene probe to ensure that equal amounts of RNA were present on the membrane during hybridization with the respective probes.

2.5. Thylakoid preparation and Western hybridization

The N-phase and P-phase cells were harvested, washed with HM buffer (50 mM HEPES–NaOH,

Table 1
DNA probes used in this study

Source organism	Source plasmid	Gene	Gene product	Fragment used as probe	Reference
Synechocystis 6803	pKW1266	*psb*A	D1	0.5 kb *Kpn*I-*Hin*dIII	[36]
Synechococcus 7942	pAM011	*psb*D	D2	1.3 kb *Bam*HI	[37]
Synechococcus 7942	pGS102	*psb*C	CP43	0.4 kb *Pst*I-*Hin*dIII	[37]
Anabaena 7120	pAN621	*rrn*	23S rRNA	4.0 kb *Eco*RI	[38]
Anabaena 7120	pAN154.3	*nif* H	Fe protein nitrogenase	1.8 kb *Hin*dIII	[39]
Plectonema	PCR product	*psb*B	CP47	623 bp *Hin*dIII	This study

pH 7.5; 20 mM $MgCl_2$), stored at −70°C and then thawed before use. The protocols for membrane preparation, SDS–PAGE and staining with Coomassie brilliant blue R250 were as described [23]. Immunoblotting and hybridization were carried out using antiserum specific for apoprotein of CP47 [20].

3. Results

3.1. Diazotrophic growth and the excitation energy transfer from the phycobilisomes to the photosynthetic reaction centers

P. boryanum grown photoautotrophically under diazotrophic conditions showed temporal separation of oxygenic photosynthetic activity and nitrogen fixation (Fig. 1). A low level of oxygen evolution corresponded to high nitrogen fixing activity which decreased again when the oxygen evolving activity of PSII increased. We have earlier shown that N-phase cells showed low PSII and high PSI activity compared to P-phase cells [13].

77 K fluorescence emission spectra were recorded with N- and P-phase cells to study the excitation energy distribution between PSI and PSII in these two phases. Fig. 2 shows the normalized emission

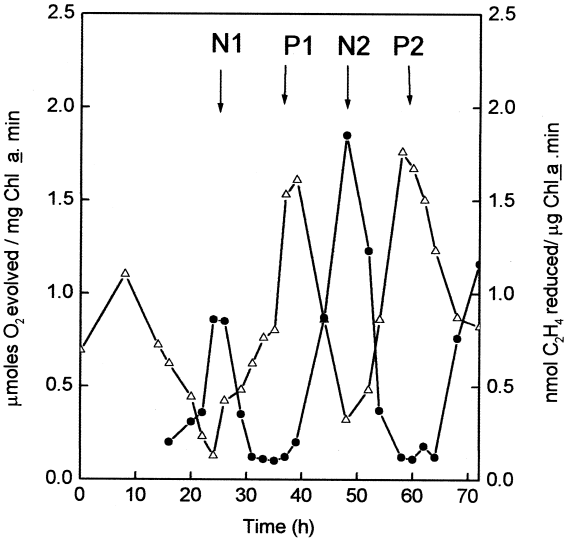

Fig. 1. Alternating cycles of nitrogen fixation (N) and photosynthesis (P) in *P. boryanum* strain UTEX 594 grown photoautotrophically under diazotrophic conditions. Acetylene reduction activity (●) and light-dependent oxygen evolution (△), monitored in the medium during the first two cycles, are plotted.

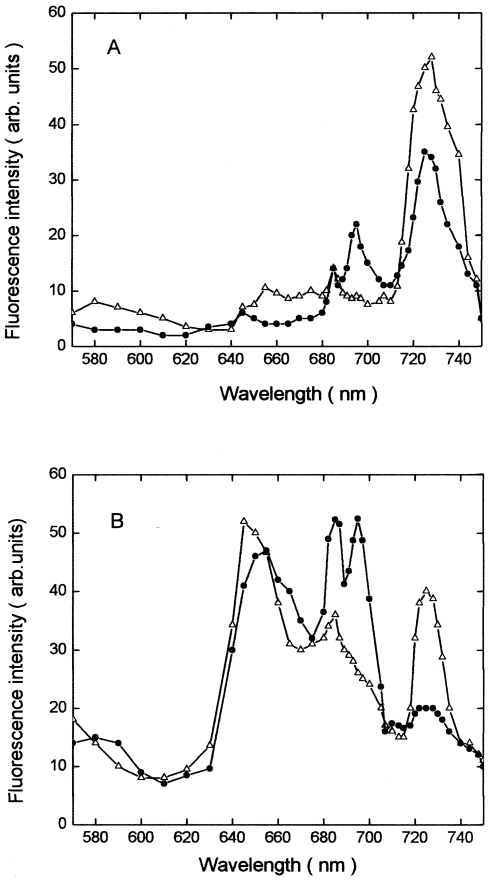

Fig. 2. Normalized 77 K fluorescence emission spectra of N-phase (△) and P-phase cells (●) of wild-type *P. boryanum* grown photoautotrophically under diazotrophic conditions. (A) Excitation at 437 nm. (B) Excitation at 546 nm. The bandwidth of the excitation and emission monochromators was 10 nm and 2.5 nm, respectively.

spectra of cells from the two phases excited at 437 and 546 nm, separately. The typical spectrum showed three major regions: (i) a broad peak at around 645–655 nm corresponding to phycobilin pigments, phycocyanin and allophycocyanin, associated with the distal antenna system; (ii) two nearby peaks at 685 and 695nm which are contributed by the Chl*a* molecules bound to CP43 and CP47, respectively [25,26] of the PSII core complex; (iii) a 725 nm peak originating from the PSI complex [27]. Comparison of the normalized 77 K fluorescence data of the N-phase and the P-phase showed that the N-phase cells exhibit (i) a higher yield of $F_{725\,nm}$, (ii) the absence of the $F_{695\,nm}$ peak, and (iii) a blue shift of the phycocyanin emission peak from $F_{650\,nm}$ in the P-phase to $F_{645\,nm}$ in the N-phase. These results sug-

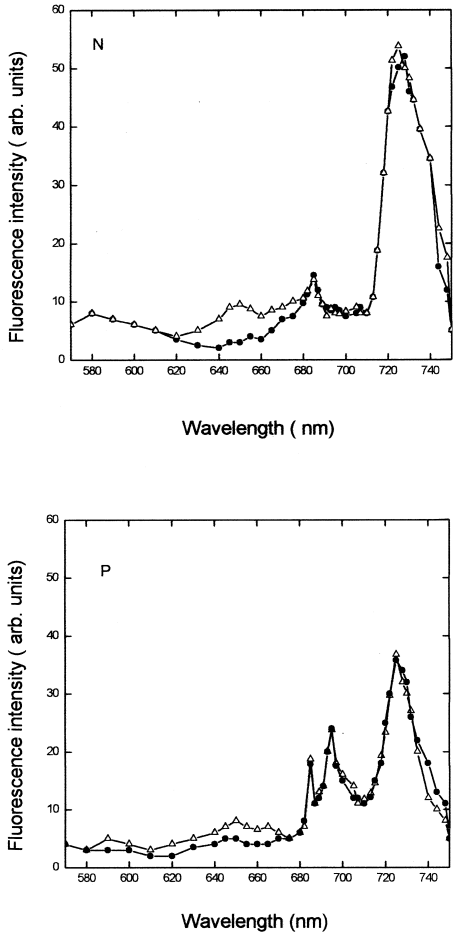

Fig. 3. Normalized 77 K fluorescence spectra of intact cells (△) and thylakoids (●) from N-phase (N) and P-phase (P) cells. Samples were illuminated under simulated conditions of N- and P-phase and frozen in liquid nitrogen. The emission spectra were recorded at 437 nm excitation and normalized at 685 nm. The bandwidth of the excitation and emission monochromators was 10 nm and 2.5 nm, respectively.

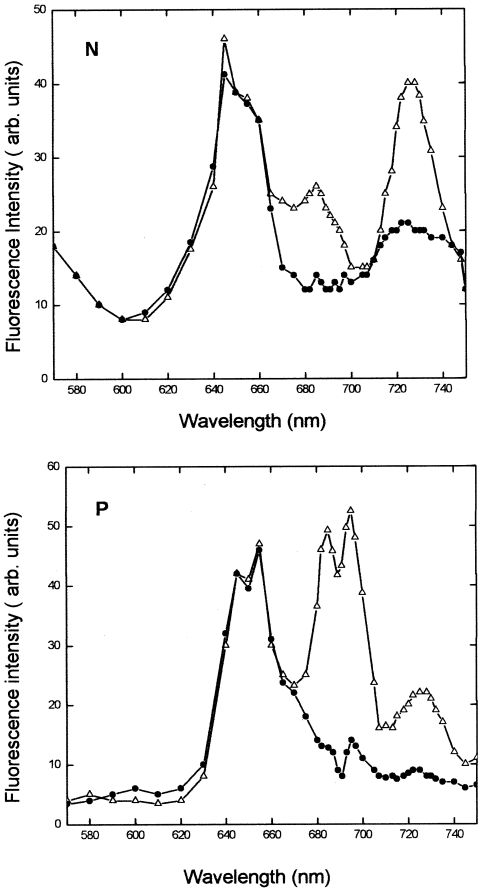

Fig. 4. Normalized 77 K fluorescence spectra of intact cells (△) and thylakoids (●) from N-phase (N) and P-phase (P) cells. Samples were illuminated under simulated conditions of N- and P-phase and frozen in liquid nitrogen. The emission spectra were recorded at 546 nm excitation and normalized at 650 nm. The bandwidth of the excitation and emission monochromators was 10 nm and 2.5 nm, respectively.

gested that the N- and P-phase cells have different modes of excitation energy transfer from PBS to photosystems. Specifically the P-phase cells had spectral characteristic of state 1 light adaptation and the N-phase cells of state 2 adaptation. The ratio of the heights of the PSI peak at 726 nm in the spectra excited at 546 nm and 437 nm has been considered

Table 2
Excitation energy distribution from PBS to photosystems in intact cells and thylakoids prepared from N- and P-phase cells of *Plectonema*

Growth condition	Treatment	F_{685}/F_{726} at excitation		F_{726} (546)/F_{726} (437)	F_{685} (546)/F_{685} (437)
		546 nm	437 nm		
N-phase	Control	0.95	0.28	0.76	2.57
	Thylakoids	0.67	0.313	0.41	0.87
P-phase	Control	2.65	0.40	0.57	3.71
	Thylakoids	1.42	0.39	0.19	0.67

The data presented here are from Figs. 2–4.

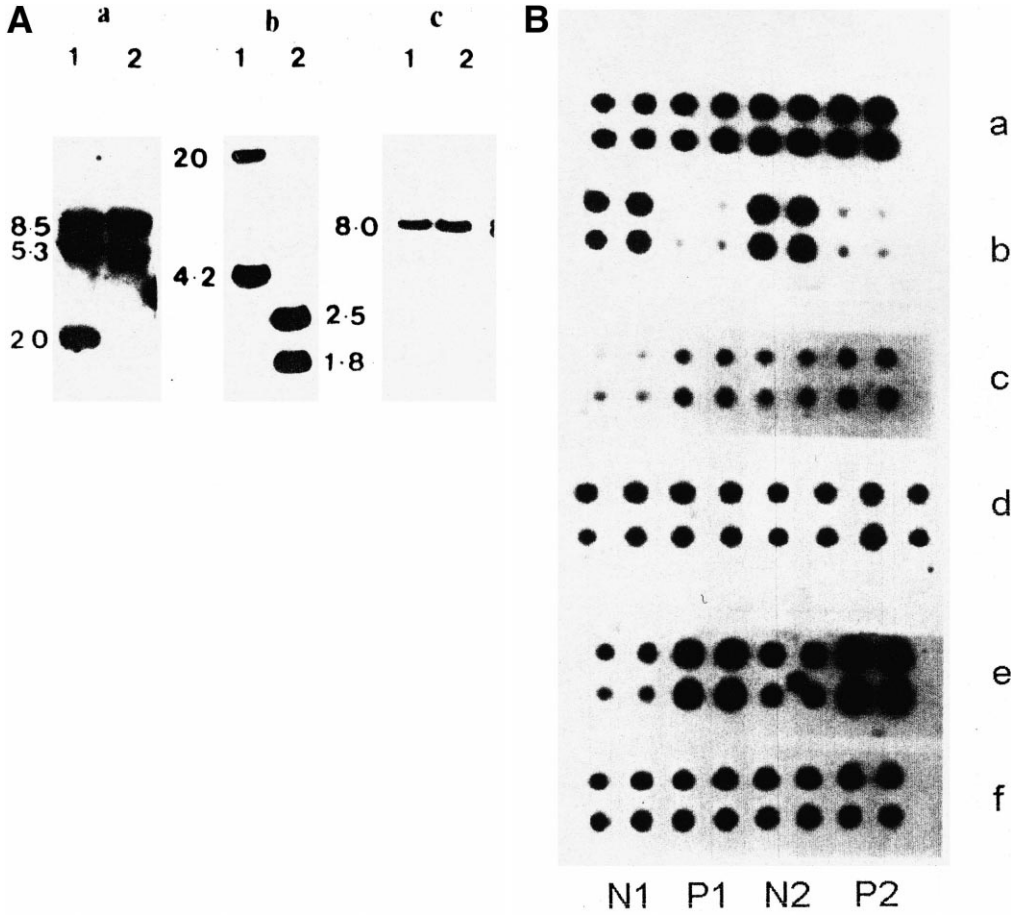

Fig. 5. Detection and expression of PSII component genes from *P. boryanum*. (A) Southern hybridization of total genomic DNA digested with *Hin*dIII (1) and *Eco*RI (2) using *psb*A (a), *psb*D (b), and *psb*C (c) gene probes. Sizes of the fragments hybridized with the respective probes are given in kb. (B) RNA dot blot hybridization of RNA prepared from two subsequent N-phases (N1 and N2) and P-phases (P1 and P2) blotted in quadruplicate and hybridized with *rrn* (a), *nif*H (b), *psb*A (c), *psb*B (d), *psb*C (e), and *psb*D (f) gene probes as described in Section 2.

an indicator of the efficiency of energy transfer from PBS to PSI [12]. This ratio was 0.57 in P-phase cells and 0.76 in N-phase cells indicating that during N-phase the proportional energy transfer from PBS to PSI increases.

In cyanobacteria, a considerable contribution to the fluorescence emission between 630 and 670 nm comes from the phycobilin pigments bound to the distal antenna system. These get detached from the photosystems during the normal process of thylakoid preparation. In intact cell fluorescence the phycobilin emission cross-contaminates the PSII emission coming from the 685 nm peak. Therefore, the F_{685} peak in the N-phase, though very poorly, could be due to contamination from the phycobilin pigments. To ascertain this, the thylakoids were prepared from the N- and P-phase cells and low temperature fluorescence spectra were recorded at 437 and 546 nm separately. Fig. 3 presents the 77 K emission spectra of thylakoid and intact cells normalized at the F_{685} peak. Only the F_{685} peak was present in the thylakoids of the N-phase cells while the P-phase thylakoid showed the F_{685} and the F_{695} peaks on chlorophyll excitation (437 nm) (Fig. 3). Both of these peaks disappeared from the thylakoids in both phases while the F_{725} yield showed a significant decrease on excitation of the phycocyanin (546 nm) (Fig. 4). Characteristically, a blue shift in the phycocyanin emission maxima from 650 nm to 645 nm as compared to intact cells was observed with the P-phase thylakoid preparations (Fig. 4P). The emission spectra recorded at 437 nm showed similar spectral

characteristics and proportionate yields for the F_{685} and F_{726} peaks in both thylakoids and cells (Table 2). These results are consistent with our hypothesis that during thylakoid preparation loosely bound water-soluble PBS get detached from the photosystems leading to the disappearance of F_{685}/F_{695} and a decrease in the F_{726} emission. The lack of the 695 nm peak and the consistent presence of the F_{685} peak in both the thylakoid and whole cells of the N-phase at 437 nm excitation indicated that the chlorophyll binding protein CP47 was not assembled in the PSII reaction center in these cells.

3.2. Expression of photosynthetic genes in the N- and P-phases

Southern hybridization was carried out with DNA isolated from *P. boryanum* using *psb*A, *psb*C and *psb*D DNAs as probes as given in Table 1. The results obtained from Southern hybridization demonstrated conservation of the sequences of these genes among different cyanobacterial systems and the specificity of heterologous probes used in this study (Fig. 5a). Using these probes, RNA dot blot hybridization was carried out with RNAs prepared from the N- and P-phases. Results presented here showed that the *psb*D and *psb*B transcript levels were comparable in these phases while *psb*A and *psb*C decreased in the N-phase compared to the P-phase (Fig. 5b) and the *nif*H transcript showed a trend reciprocal to that of *psb*A and *psb*C. Hybridization of RNA dot blot with the *rrn* gene probe showed that approximately equal amounts of total RNA were present in each dot.

The near equality between the *psb*B transcript level in the N- and P-phases is intriguing in view of the absence of the 77 K fluorescence peak at F_{695} in the N-phase. To resolve this apparent contradiction we determined the level of CP47 in the thylakoid protein from the N- and P-phase cells. The thylakoid proteins isolated from the N- and P-phase cells were separated on SDS–PAGE and transferred to polyvinylidene fluoride membranes. The blots were hybridized with antibodies against CP47 apoprotein. The results presented in Fig. 6 show that the CP47 antibody hybridized with two very similarly sized protein bands in the P-phase thylakoids (lane 2) while it gave a very poor signal in the N-phase thylakoids (lane 1).

Fig. 6. Western blot analysis of thylakoid proteins from N-phase (lane 1) and P-phase (lane 2) cells of *Plectonema* grown photoautotrophically under diazotrophic conditions using antibodies against CP47 apoprotein.

The reason for the presence of the two bands in lane 2 is not clear at this stage. The highly unlikely possibility of our anti-CP47 serum having antibody impurity that recognizes another thylakoid protein and the possibility of CP47 being present in two forms have not been completely ruled out. However, it is obvious that in spite of the normal level of the *psb*B mRNA the N-phase thylakoids have little CP47, if any. Whether this is due to poor translation of the *psb*B mRNA or poor incorporation of CP47 into thylakoids in N-phase cells will be interesting to investigate.

4. Discussion

P. boryanum grown photoautotrophically under diazotrophic conditions shows alternate phases of photosynthesis and nitrogen fixation [13,28]. During the N-phase, the activity of PSII that is responsible for oxygen evolution is suppressed and the PSI activity increases by 40–50%. This is perhaps required

for the organism to perform light-dependent nitrogen fixation [16,29]. The uncoupling of the PSI activity from PSII can be explained if we postulate either that during N-phase the PSI reaction centers get light energy directly from the light harvesting antenna pigments and/or that during this phase oxidized P700 can be reduced either by cyclic electron flow from $NADPH_2$ via PQ [30,31] or by oxidation of endogenous electron donors like glycollate in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea [16]. The present study was carried out to elucidate the mode of light energy transfer from the PBS to PSI in the absence of the PSII activity during the N-phase. The 77 K fluorescence spectra excited at 546 nm revealed that the N- and P-phase cells might have different pathways of energy transfer from PBS to PSI (Fig. 2). On excitation at 437 nm the N- and P-phase cells showed spectra characteristic of state 2 and state 1, respectively. Since N-phase cells have little PSII activity [13] and an impaired electron transport between Q_A and Q_B [15], the major route of excitation energy transfer from the PBS to PSI is likely to be direct rather than a 'spillover' from PSII. This kind of alteration in the excitation energy distribution from a light harvesting antenna pigment to the photosystems has been reported to occur through state transition in photosynthetic organisms [11]. In cyanobacteria, it has been suggested that transition to state 2 involves the decoupling of a portion of the PSII core complex from PBS and the binding of the PSI core complex in its place [5,19]. In such cases, it has been shown that PBS are present as a PBS–PSI complex and could transfer the excitation energy directly to PSI in state 2-adapted *Synechococcus* PCC6801 and PSII-free mutants of *Synechococcus* PCC6803 [12]. These mutants lack the genes for the D2 and CP43 proteins and the PSII particles from such mutants were devoid of the D1 and CP47 proteins and contained unstable PSII reaction centers [32,33]. These mutated cells did not show transition to state 1 as PBS decoupling from PSI failed to enhance PSII emission [12]. Subsequently, it was shown that the mutations in CP47 that could destabilize PSII activity did not show the F_{695} emission peak in the 77 K fluorescence spectra [34]. The lack of the F_{695} emission peak (Fig. 2) and CP47 in the N-phase thylakoid (Fig. 6) suggested that the N-phase cells may have a similar PSII organization as *psb*D-C mutants of *Synechococcus* PCC6803 [12].

The decreased levels of the *psb*C transcript in the N-phase cells (Fig. 5) and of the CP47 protein in the N-phase thylakoids as compared to the P-phase cells suggest a defect in assembling of the PSII reaction center when the cells enter the nitrogen fixation phase. If we assume that in the N-phase cells the PSII assembly was analogous to the *psb*DI-C mutants of *Synechococcus* [12] then the N-phase may not be driven to state 1 transition. Results on the 77 K fluorescence of the N- and P-phase cells adapted to PSI and PSII lights showed that unlike P-phase cells the N-phase cells could not be induced to state 1 transition (data not shown). Thus it may be suggested that the presence of a stably assembled PSII reaction center determines the excitation energy distribution from the PBS to the photosystems. However, the mechanism by which the PSII activity decreases so rapidly and the cells enter the N-phase is not understood and would be interesting to investigate. One possibility could be that one or more of the metabolic changes associated with the N-phase, such as a decrease in intracellular nitrogen concentration, the levels of reducing and energy equivalents, reduction of the PQ pool, change in the redox potential of PET components, etc. [35], inhibit the availability of CP47 and CP43 for PSII assembly and regulate the specificity of PBS towards photosystems.

Acknowledgements

We thank Dr. Susan S. Golden, Prof. R. Haselkorn and Dr. C. Jansson for generous gift of plasmids pAM011 and pGS102 [37], pAN621 and pAN154.3 [38] and pKW1266 [36], respectively. We are grateful to S.K. Bhattacharjee, Dr. A.S. Bhagwat and Prof. Prassana Mohanty for their help in data interpretation and during the preparation of this article. We are also indebted to Prof. Peter Westhoff and Dr. P.V. Sane for providing anti-CP47 sera and Dr. Rakesh Tuli and Ms. Sushma Naithani (NBRI, India) for help in Western hybridization and result interpretation.

References

[1] A.N. Glazer, Biochim. Biophys. Acta 768 (1984) 29–51.
[2] D.A. Bryant, G. Guglielmi, N. Tandeau de Marsac, A.M. Castets, G. Cohen- Bazire, Arch. Microbiol. 123 (1979) 113–127.
[3] E. Gantt, C.A. Lipschuttz, Biochim. Biophys. Acta 292 (1973) 858–861.
[4] A.R. Grossman, M.R. Schaefer, G.G. Chiang, J.L. Collier, Microbiol. Rev. 57 (1993) 725–749.
[5] C.W. Mullineaux, Biochim. Biophys. Acta 1100 (1992) 285–292.
[6] W.L. Butler, M. Kilajima, Biochim. Biophys. Acta 396 (1975) 72–82.
[7] C.W. Mullineaux, A.R. Holzwarth, Biochim. Biophys. Acta 1098 (1991) 68–78.
[8] C.W. Mullineaux, S. Griebenow, S.E. Braslavsky, Biochim. Biophys. Acta 1060 (1991) 315–318.
[9] D. Bruce, J. Biggins, T. Steiner, M. Thewalt, Biochim. Biophys.Acta 806 (1985) 237–246.
[10] W.P. William, J.F. Allen, Photosynth. Res. 13 (1987) 19–45.
[11] J. Biggins, D. Bruce, Photosynth. Res. 20 (1989) 1–34.
[12] C.W. Mullineaux, Biochim. Biophys. Acta 1184 (1994) 71.
[13] H.S. Misra, R. Tuli, J. Plant Biochem. Biotechnol. 2 (1993) 101–104.
[14] W.D.P. Stewart, M. Lex, Arch. Microbiol. 73 (1970) 250–260.
[15] H.S. Misra, T.S. Desai, Biochem. Biophys. Res. Commun. 194 (1993) 1001–1007.
[16] H.S. Misra, R. Tuli, Indian J. Biochem. Biophys. 31 (1994) 310–314.
[17] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, J. Gen. Microbiol. 111 (1979) 1–61.
[18] A.K. Vacchani, R.K. Iyer, R. Tuli, J. Gen. Microbiol. 139 (1993) 569–573.
[19] J.F. Allen, C.E. Sanders, N.G. Holmes, FEBS Lett. 193 (1985) 271–275.
[20] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.
[21] R.H. Felkner, S.R. Barnum, Curr. Microbiol. 17 (1988) 37–41.
[22] P. Chomczynski, N. Sacchi, Anal. Biochem. 162 (1987) 156–159.
[23] N.H. Mann, R. Rippka, M. Herdman, J. Gen. Microbiol. 137 (1991) 331–339.
[24] S.A. Bustos, M.R. Schaefer, S.S. Golden, J. Bacteriol. 172 (1990) 1998–2004.
[25] H.Y. Nakatani, B. Key, E. Dolan, C.J. Arntzen, Biochim. Biophys. Acta 765 (1984) 347–352.
[26] R.J. van Dorssen, J. Breton, J.J. Plijter, K. Satoh, H.J. van-Gorkom, J. Amesz, Biochim. Biophys. Acta 893 (1987) 267–274.
[27] M. Rogner, P.J. Nixon, B.A. Diner, J. Biol. Chem. 265 (1990) 6189–6196.
[28] N.M. Weare, J.R. Benemann, J. Bacteriol. 119 (1974) 258–265.
[29] H.S. Misra, R. Tuli, Microbiology 140 (1994) 971–976.
[30] H. Mi, T. Endo, U. Schreiber, T. Ogawa, K. Asada, Plant Cell Physiol. 35 (1994) 163–173.
[31] H.C.P. Matthijs, E.M.E. Luderus, M.J.C. Scholts, R. Kraayenhof, Biochim. Biophys. Acta 766 (1984) 38–44.
[32] W.F.J. Vermaas, M. Ikeuchi, Y. Inoue, Photosynth. Res. 17 (1988) 97–113.
[33] W.F.J. Vermaas, J. Charite, B. Eggers, in: M. Baltscheffsky (Ed.), Current Research in Photosynthesis, Vol. 1 Kluwer, Dordrecht, 1990, pp. 231–238.
[34] E. Haag, J.J. Eaton-Rye, G. Renger, W.F.J. Vermaas, Biochemistry 32 (1993) 4444–4454.
[35] R. Tuli, S. Naithani, H.S. Misra, J. Sci. Ind. Res. 55 (1996) 638–657.
[36] C. Jansson, R.J. Debus, H.D. Osiewacz, M. Gurevitz, L. McIntosh, Plant Physiol. 85 (1987) 1021–1025.
[37] S.S. Golden, G.W. Stearn, Gene 67 (1988) 85–96.
[38] S.A. Nierzwicki-Bauer, R. Haselkorn, EMBO J. 5 (1986) 29–35.
[39] M. Mevarech, D. Rice, R. Haselkorn, Proc. Natl. Acad. Sci. USA 77 (1980) 6476–6480.