

BBABIO 43035

## In vivo phosphorylation of proteins in the cyanobacterium *Synechococcus* 6301 after chromatic acclimation to Photosystem I or Photosystem II light

Christine E. Sanders, Anastasios Melis\* and John F. Allen

Department of Pure and Applied Biology, University of Leeds, Leeds (U.K.)

(Received 23 March 1989)

Key words: Photosynthesis; Thylakoid membrane; Phycobilisome; Photosystem stoichiometry; Protein phosphorylation; (Cyanobacterium)

Cells of the cyanobacterium *Synechococcus* 6301 were grown in the presence of  $^{32}\text{P}$ -orthophosphate under light absorbed preferentially by Photosystem I (PS I light), or by Photosystem II (PS II light). The acclimation of cells to these light regimes resulted in a higher phycobilisome-PS II/PS I ratio under PS I light than under PS II light. Under steady-state cell growth, the in vivo phosphorylation of four distinct polypeptides (20, 19, 15 and 13 kDa) was observed. In the membrane fraction, a 15 kDa protein was phosphorylated more under PS II light than under PS I light. In the soluble fraction, a 13 kDa protein was phosphorylated exclusively under PS II light. In the phycobilisome fraction, a 19 kDa protein was phosphorylated more under PS II light. The possible role of differential protein phosphorylation in the chromatic acclimation (photosystem stoichiometry adjustment) of cells is discussed.

### Introduction

There is a wide variation in photosystem stoichiometry (PS II/PS I ratio) among different photosynthetic organisms [1]. In sun-adapted higher plant chloroplasts, such as those of pea or spinach, the PS II/PS I ratio is 1.8 [2,3]. In cyanobacteria, however, the PS II/PS I ratio is usually much lower, a typical value being 0.5 [1,4,5]; furthermore the chlorophyll *a* antenna in each PS I centre is about 3.5 times the size of that in each PS II centre. This imbalance in chlorophyll light-harvesting capacity between the two photosystems is corrected by the association of PS II with the phycobilisome. The phycobilisome (PBS) is a large, extrinsic, pigment-protein complex which contains 350–400 light-harvesting phycobilin chromophores [6,7].

The PS II/PS I ratio in both cyanobacteria and higher plants is not fixed; it can be adjusted to optimise the efficiency of photosynthesis in response to different

growth conditions. Several investigators have reported that prolonged exposure to either PS I or PS II light causes a substantial long-term change in the PS II/PS I ratio which, in cyanobacteria, can be measured most easily as a change in the relative content of phycobilins and chlorophyll [5,8,9]. Cells grown in yellow light, which preferentially excites the PBS-PS II complex, develop a low phycobilin/chlorophyll ratio. Cells grown in red light, which preferentially excites PS I, develop a high phycobilin/chlorophyll ratio. These studies indicated that cyanobacteria, much like higher plant chloroplasts, possess a feedback control mechanism which enables them to regulate the synthesis/assembly of PBS-PS II and PS I complexes in order to balance the utilisation of excitation energy by the two photoreactions. It was reported [5,8,9] that the observed changes in PS stoichiometry were sufficient to equalise the overall absorption of light of PS II and PS I except in the case of *Synechococcus* 6301 grown under phycobilisome excitation [8].

The phosphorylation properties of a 15 kDa protein in the membrane fraction and of an 18.5 kDa protein in the phycobilisome fraction of *Synechococcus* 6301 were reported [10–12]. Here we report on the steady-state phosphorylation of these proteins in vivo after long-term acclimation of cells to PS I or PS II light. We also report on a novel phosphoprotein, of about 13 kDa, whose in vivo phosphorylation is dependent on long-

\* Permanent address: Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720, USA. Abbreviations: Chl, chlorophyll; PBS, phycobilisome; PS, Photosystem;  $\text{P}_i$ , inorganic orthophosphate; TCA, trichloroacetic acid.

Correspondence: J.F. Allen, Department of Pure and Applied Biology, University of Leeds, Leeds, LS2 9JT, U.K.

term acclimation of the *Synechococcus* cells to PS II light.

### Experimental

*Synechococcus* 6301 (UTEX 625) was grown photoautotrophically at 35°C in medium C of Kratz and Myers [13] except that the orthophosphate concentration was reduced to 0.4 mM which is 10% of the normal concentration (10% P<sub>i</sub> medium C). The cultures were continuously stirred and bubbled with 5% CO<sub>2</sub> in nitrogen. Illumination was provided either by cool white fluorescent lights in combination with a yellow cut-off filter (Cinemoid Orange 5, Strand Lighting, Isleworth, London, U.K.) which preferentially excites phycobilisome pigments (PS II light) or by tungsten filament bulbs in combination with a far-red cut-off filter (Chromoid Ruby 114, Strand Lighting) which preferentially excites chlorophyll *a* (PS I light). The intensities of the two lights were adjusted to give about equal rates of cell growth in the two cultures.

*Synechococcus* 6301 cells were grown under PS I and PS II light for several days until the light acclimation, as judged by changes in the absorption spectra of the cells, was complete. During the acclimation, cells were maintained in early to mid-log phase by dilution with fresh 10% P<sub>i</sub> medium C. Cells from 200 ml of mid-log phase culture were harvested by centrifugation under sterile conditions and resuspended in 400 ml of orthophosphate-free medium C (P<sub>i</sub>-free medium C). The PS I- and PS II light cultures were adjusted, by the addition of P<sub>i</sub>-free medium C, to equal cell density estimated by light scattering at 750 nm, <sup>32</sup>P-orthophosphate was then added to give 5 μCi/ml and the cells were grown overnight (approx. 20 h).

Sphæroplasts were prepared as in Ref. 11 except that the cells were resuspended in 40 ml lysozyme buffer with lysozyme at 10 mg·ml<sup>-1</sup> and incubated for 30 min. During the lysozyme treatment the samples were illuminated with PS I or PS II light as appropriate, 1 ml aliquots of the spheroplasts were lysed, as in Ref. 11, to give the membrane and soluble fractions from which the proteins were immediately precipitated by the addition of trichloroacetic acid (TCA) to 5%. The remaining spheroplasts were harvested by centrifugation (10 000 × *g* for 10 min) then lysed by homogenisation in 1 M Na/KPO<sub>4</sub> buffer (pH 7.5) [14] with 1% (v/v) Triton X-100. Intact phycobilisomes were isolated as in Ref. 14 except that lysis of the spheroplasts replaced breaking the cells in the French press. The intact phycobilisome preparation was separated from Triton-solubilised chlorophyll by passing the sample through a small hydroxylapatite column (1 × 2 cm) equilibrated with 10% glycerol, 0.6 M Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0). The phycobiliproteins were precipitated by the addition of TCA to 5%.

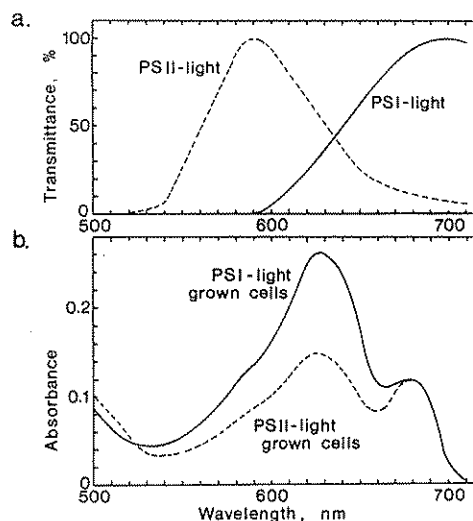


Fig. 1. (a) The emission spectra of the light sources designed to excite preferentially the phycobilisome (PS II light) or chlorophyll (PS I light). The transmittance spectra were normalised to 100% at the wavelength of maximum emission. (b) Absorbance spectra of *Synechococcus* 6301 cells acclimated to PS I light or to PS II light. The absorbance spectra of the two cell types were normalised to the chlorophyll maximum (678 nm).

After TCA precipitation, all samples were extracted with 80% acetone to remove lipid then cooled to -20°C to precipitate protein. The extraction was repeated until the 80% acetone extract was clear; at least three changes of 80% acetone were used for the membrane fraction samples. Samples were solubilised with SDS, treated with RNase and run on SDS-PAGE as in Ref. 11. Phosphorylated polypeptides were detected by autoradiography using Amersham Hyperfilm MP.

All reagents were Analar or equivalent. Enzymes were from Sigma and radioisotopes were from New England Nuclear.

### Results

Fig. 1a shows the emission profiles of the broad band yellow and red lights designed to excite preferentially either the phycobilisome (PS II light) or chlorophyll (PS I light). The absorbance spectra of *Synechococcus* cells grown under PS I and PS II light are shown in Fig. 1b. The PS I light grown cells have a much higher ratio of absorbance at 625 nm to absorbance at 678 nm ( $A_{625}/A_{678}$ ) than the PS II light grown cells; the  $A_{625}/A_{678}$  is typically approx. 2.2 for PS I light grown cells and approx. 1.3 for PS II light grown cells. The higher  $A_{625}/A_{678}$  in cells grown under PS I light reflects a higher phycobilin to chlorophyll ratio. This increase in the proportion of phycobilins is due to an increase in the number of PBS-PS II complexes as compared to PS I complexes rather than an increase in the rod-size of individual phycobilisomes [5,8,15].

We have examined the phosphorylation state of polypeptides from PS I and PS-II light grown cells of

*Synechococcus* 6301. We have used SDS-PAGE and autoradiography to indicate the extent of phosphorylation of polypeptides from membrane, soluble and intact phycobilisome fractions of the cells. The membrane fraction was pelleted by centrifugation after osmotic lysis of spheroplasts. Tracks from SDS-PAGE of proteins from the membrane fraction of *Synechococcus* 6301 cells acclimated to PS I light or PS II light are shown in Fig. 2a; Fig. 2b shows the autoradiograph of Fig. 2a. Four phosphorylated polypeptides can be seen: two major phosphorylated polypeptides at about 20 and 15 kDa (judged by comparison with known molecular weights of the marker proteins) and two minor ones at about 19 and 13 kDa. Both the 15 kDa and the 20 kDa polypeptides show greater  $^{32}\text{P}$ -incorporation in cells grown in PS II light compared to PS I light grown cells; however, the difference in  $^{32}\text{P}$ -incorporation into the 15 kDa polypeptide between the two cell types is slightly more marked. Of the two minor phosphopolypeptides that at 19 kDa also shows greater  $^{32}\text{P}$ -incorporation in PS II light cells and the 13 kDa polypeptide appears to be phosphorylated exclusively in the PS II light grown cells.

The membrane fraction shown in Fig. 2 does not contain a significant proportion of the phycobiliproteins, which dissociate from the membranes on osmotic lysis of the spheroplasts. On centrifugation of the lysed spheroplasts the phycobiliproteins appear in the supernatant which forms the soluble fraction of the

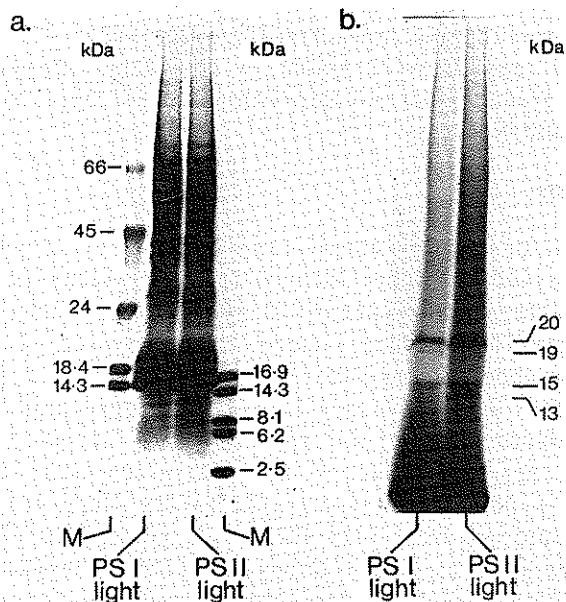


Fig. 2. Membrane fraction proteins of *Synechococcus* 6301 cells acclimated to either PS I-light or PS II-light. (a) SDS-PAGE tracks loaded on the basis of equal cell density. Tracks labelled M are molecular-mass markers: the molecular mass of the markers in kDa is shown alongside the gel. (b) Autoradiograph of the SDS-PAGE tracks shown in (a). The estimated molecular masses of the phosphorylated polypeptides in kDa are shown alongside the autoradiograph.

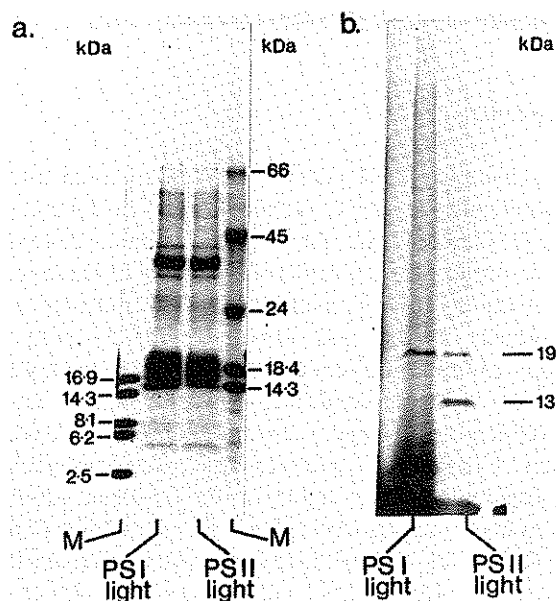


Fig. 3. Soluble fraction proteins of *Synechococcus* 6301 cells acclimated to either PS I light or PS II light. (a) SDS-PAGE tracks loaded on the basis of equal cell density. Tracks labelled M are molecular-mass markers: molecular mass of the markers in kDa is shown alongside the gel. (b) Autoradiograph of the SDS-PAGE tracks shown in (a). The estimated molecular masses of the phosphorylated polypeptides in kDa are shown alongside the autoradiograph.

cells. Fig. 3 shows SDS-PAGE (Fig. 3a) and autoradiography (Fig. 3b) of the proteins from the soluble fraction of *Synechococcus* 6301 cells acclimated to PS I or PS II light. The two minor phosphorylated polypeptides (19 and 13 kDa) of the membrane fraction are seen to be the major phosphorylated components of the soluble fraction (Fig. 3b). As in the membrane fraction (Fig. 2), the 13 kDa polypeptide in the soluble fraction is phosphorylated only in cells grown under PS II light (Fig. 3). However, unlike in the membrane fraction (Fig. 2), the 19 kDa polypeptide is seen to be phosphorylated in the soluble fraction of cells grown both under PS I and under PS II light (Fig. 3). Furthermore, there is a greater  $^{32}\text{P}$ -incorporation into the 19 kDa polypeptide in the soluble fraction of PS I light cells (Fig. 3).

Although the phycobiliproteins are the principal protein component in the soluble fraction it also contains proteins which are not associated with the phycobilisomes. To identify which, if either, of the phosphorylated polypeptides seen in the soluble fraction is associated with the phycobilisomes, we isolated an intact phycobilisome fraction from the cells. Tracks from SDS-PAGE of proteins from the intact phycobilisome fraction of *Synechococcus* 6301 cells grown under PS I or PS II light are shown in Fig. 4a and the autoradiograph of these tracks shown in Fig. 4b. Only one polypeptide, of about 19 kDa, is phosphorylated in the intact phycobilisome fraction and the  $^{32}\text{P}$ -incorporation into this polypeptide is greater in phycobilisomes from

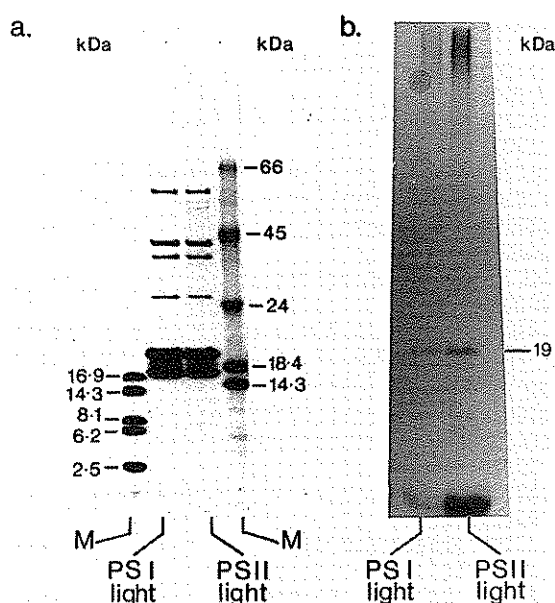


Fig. 4. Intact phycobilisome fraction proteins of *Synechococcus* 6301 cells acclimated to either PS I light or PS II light. (a) SDS-PAGE tracks loaded on the basis of equal phycobilin concentration. Tracks labelled M are molecular-mass markers: the molecular mass of the markers in kDa is shown alongside the gel. (b) Autoradiograph of the SDS-PAGE tracks shown in (a). The estimated molecular mass of the phosphorylated polypeptide in kDa is shown alongside the autoradiograph.

PS II-light grown cells than in those from PS I-light grown cells.

## Discussion

The results from SDS-PAGE and autoradiography presented in this work reveal the steady-state phosphorylation pattern of several polypeptides in *Synechococcus* 6301 acclimated to PS I or PS II light. In the membrane fraction there are distinct phosphoproteins of 20 and 15 kDa. Of the two, the 15 kDa polypeptide appears to be phosphorylated to a greater extent in cells grown under PS II light conditions than in cells grown under PS I light conditions. The presence of small amounts of the 19 kDa and 13 kDa phosphoproteins in the membrane fraction (Fig. 2) may suggest either that these polypeptides are loosely bound membrane proteins that become dissociated upon lysis of the spheroplasts, or that they represent a minor contamination of the membrane fraction with soluble protein. The principal protein components of the soluble phase, the phycobiliproteins, are loosely bound to the membranes and dissociate upon lysis of the cells.

In the soluble fraction, distinct phosphoproteins of 19 and 13 kDa are differentially phosphorylated under PS I light and PS II light conditions. The 19 kDa polypeptide (probably a constituent of the PBS) appears to contain more label in the PS I light grown cells. This

result could arise because the tracks on the gel of the soluble fraction (Fig. 3a) are loaded on the basis of equal cell density. PS I light grown cells have a much greater number of phycobilisomes per cell than those grown in PS II light [5,8,15]. Since phycobiliproteins are the major component of the soluble fraction, the results could suggest a greater PBS loading rather than a greater  $^{32}\text{P}$ -incorporation into the 19 kDa polypeptide in PS I light cells. In intact phycobilisomes (loaded on the basis of equal phycobiliprotein concentration in Fig. 4), the 19 kDa polypeptide shows greater labelling in PS II light grown cells, suggesting a greater  $^{32}\text{P}$ -incorporation into this polypeptide. The apparent increase in phosphorylation of the 19 kDa polypeptide in the soluble fraction of PS I light grown cells is perhaps greater than would be expected from the compensating effects of increased  $^{32}\text{P}$ -incorporation into the 19 kDa polypeptide in PS II light grown cells and increased phycobiliprotein concentration in the soluble fraction of PS I light grown cells. Furthermore, if the apparent increase in phosphorylation of the 19 kDa polypeptide seen in the soluble fraction of PS I light grown cells were only due to the increased phycobilisome content of these cells, then a similar apparent increase should be seen in the 19 kDa polypeptide in the membrane fraction. As can be seen from Fig. 2 this is not the case. The increase in the phosphorylated 19 kDa polypeptide seen in the soluble fraction of PS I light grown cells could be enhanced by some difference, between the two cell types, in the degree of dissociation of the phycobilisomes from the membranes during lysis.

The 13 kDa polypeptide of the soluble fraction is phosphorylated exclusively in the PS II light grown cells. It is clear that this 13 kDa polypeptide is not a constituent of the PBS, since it does not appear in the intact PBS fraction. The increase in phosphorylation of this polypeptide in PS II-light grown cells could in principle be due either to an increased concentration of the polypeptide or to an increase in the specific  $^{32}\text{P}$ -incorporation. However, it is clear from the SDS-PAGE (Fig. 3) that no significant change in the concentration of this polypeptide occurs.

Earlier work investigated light-induced changes in the phosphorylation of *Synechococcus* 6301 polypeptides in vivo. It was found that phosphorylation of a membrane-bound 15 kDa polypeptide [10,11], and that of an 18.5 kDa protein associated with the PBS [12], was enhanced upon illumination with light which excited preferentially PS II. These polypeptides were dephosphorylated in PS I light. Such changes in protein phosphorylation in vivo were correlated with short-term changes in the organizations of the light-harvesting apparatus in cyanobacteria, known as state transitions [16,17]. The results reported here show the same phosphorylation pattern for these two polypeptides after the long-term acclimation of cells to PS I or PS II light. In

addition, they reveal the presence of a 13 kDa polypeptide which appears to be phosphorylated exclusively under long-term PS II light conditions.

The identity of the phosphoproteins discussed in this work is not yet known. The differential phosphorylation of the 13, 15 and 19 kDa polypeptides could be implicated in the mechanism of state transitions [10-12]; however, it appears to be a direct consequence of cell acclimation to PS I or PS II light. The persistent difference in the phosphorylation level of these polypeptides, occurring even after a long-term exposure of cells to PS I or PS II light, may suggest the involvement of the protein phosphorylation phenomenon in the chromatic acclimation of the thylakoid membrane in cyanobacteria. Since the end result of this chromatic acclimation is adjustment in photosystem stoichiometry in the thylakoid membrane, we propose a role for protein phosphorylation in this regulatory process. The identity of the 13 kDa polypeptide and the kinetics of its phosphorylation in response to long-term exposure of *Synechococcus* 6301 cells to PS II light are currently under investigation.

#### Acknowledgements

This work was supported by a SERC Research Grant to J.F.A. and by a SERC Visiting Fellowship to A.M. We thank Mr. A. Holliday for photographic work.

#### References

- 1 Melis, A. and Brown, J.S. (1980) Proc. Natl. Acad. Sci. USA 77, 4712-4716.
- 2 Melis, A. and Harvey, R.W. (1981) Biochim. Biophys. Acta 37, 138-145.
- 3 Melis, A., Manodori, A., Glick, R.E., Ghiradi, M.L., McCauley, S.W. and Neale, P.J. (1985) Physiol. Veg. 23, 757-765.
- 4 Kawamura, M., Mimuro, M. and Fujita, Y. (1979) Plant Cell. Physiol. 20, 697-705.
- 5 Myers, J., Graham, J.R. and Wang, R.T. (1980) Plant Physiol. 66, 1144-1149.
- 6 Lundell, D.J. and Glazer, A.N. (1983) J. Biol. Chem. 258, 894-901.
- 7 Manodori, A., Alhadef, M., Glazer, A.N. and Melis, A. (1984) Arch. Microbiol. 139, 117-123.
- 8 Manodori, A. and Melis, A. (1986) Plant Physiol. 82, 185-189.
- 9 Fujita, Y., Ohki, K. and Murakami, A. (1985) Plant Cell Physiol. 26, 1541-1548.
- 10 Sanders, C.E., Holmes, N.G. and Allen, J.F. (1986) Biochem. Soc. Trans. 4, 66-67.
- 11 Allen, J.F., Sanders, C.E. and Holmes, N.G. (1985) FEBS Lett. 193, 271-275.
- 12 Sanders, C.E. and Allen, J.F. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 761-764, Martinus Nijhoff, Dordrecht.
- 13 Kratz, W.A. and Myers, J. (1955) Am. J. Bot. 42, 282-287.
- 14 Grossman, A. and Brand, J. (1983) Carnegie Institution Annual Report of the Director, Department of Plant Biology, 1982-1983, pp. 116-120.
- 15 Fujita, Y. and Murakami, A. (1988) Plant Cell. Physiol. 29, 305-311.
- 16 Bonaventura, C. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366-383.
- 17 Murata, N. (1969) Biochim. Biophys. Acta 172, 242-251.