Modification of a glnB-like gene product by photosynthetic electron transport in the cyanobacterium *Synechococcus* 6301

Michael A. Harrison, Jeffrey N. Keen+, John B.C. Findlay+ and John F. Allen*

Department of Pure and Applied Biology and + Department of Biochemistry, The University of Leeds, Leeds LS2 9JT, UK

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Covalent modification of a 13 kDa soluble-phase protein occurs during adaptation of cells of the cyanobacterium *Synechococcus* 6301 (mutant AN112) to light specifically absorbed by photosystem II. This adaptation is accompanied by functional changes indicative of altered excitation energy distribution between the photosystems. The 13 kDa protein is identified by solid-phase N-terminal sequencing as a protein related to P_{II}, the glnB gene product of *E. coli*. In *E. coli*, the P_{II} protein undergoes uridylylation and acts as a regulator of glutamine synthetase at both the post-translational and transcriptional levels. The implications of modification of a transcriptional regulator by photosynthetic electron transport are discussed.

Photosynthesis; Post-translational modification; Glutamine synthetase; Transcriptional regulator; Electron transport; Redox control; *Synechococcus* 6301

1. INTRODUCTION

Post-translational modification by protein phosphorylation is known to alter functional properties of chloroplast thylakoid membrane proteins involved in light-harvesting in photosynthesis [1–3]. An imbalance in photosystem turnover induced by preferential excitation of PS II relative to PS I causes net reduction of plastoquinone. This results in activation of a redox-sensitive protein kinase, whereupon the chloroplast light-harvesting complex LHC II becomes phosphorylated and dissociates from the reaction centre core. This decreases the absorption cross-section of PS II and redistributes excitation energy away from PS II [4].

Covalent modification involving phosphate group transfer accompanies similar functional changes in the cyanobacteria [5–7]. In the cyanobacteria it is possible rigorously to define wavelength bands specific to PS I and PS II because of the distinctive phycobilin light-harvesting system of PS II with an absorption band complementary to that of the chlorophyll light-harvesting system of PS I. Growth of cells of the cyanobacterium *Synechococcus* 6301 under orange light, preferentially absorbed by the phycobilisome and therefore by PS II (Light 2), has been shown to result in phosphorylation of a thylakoid membrane protein of M_r 15000 [4,7] and of soluble proteins of 13000 [7] and 18500–19000 [4,7]. The latter is a putative phycobilisome component [8]. Phosphorylation is greatly reduced in cells grown under red light preferentially absorbed by PS I (Light 1). In this report we demonstrate that cells of *Synechococcus* 6301 (mutant AN112) performing a shorter-term adaptation to Light 2 show functional changes indicative of altered excitation energy distribution, and also increased phosphorylation of the 13 kDa and 18.5–19 kDa proteins. We also describe the purification of the 13 kDa protein and its identification by N-terminal sequencing.

Correspondence address: M.A. Harrison, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720, USA

* *Present address:* Department of Biology, University of Oslo, Box 1045, Blindern, N-0316 Oslo 3, Norway

Abbreviations: PS II, photosystem II; PS I, photosystem I; LHC II, light-harvesting chl *a*/*b*-binding protein of PS II; M_r, relative molecular mass; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

Cells of *Synechococcus* 6301 (mutant AN112 [9]) were grown to late log phase as in [7] and radiolabelled with [^{32}P]orthophosphate by 10-fold concentration into fresh, phosphate-free growth medium prior to addition of the [^{32}P]orthophosphate to an activity of 10 μCi·ml^{-1}. Cells were removed immediately to light regimes defined by orange (L2) or red (L1) filters as described in [7]. After 30 min of incubation, whole cell proteins were extracted and prepared for SDS-PAGE as in [4]. Alternatively, cells were prepared for 77 K fluorescence spectroscopy as in [5].

For isolation of the 13 kDa protein, 10 l of culture were grown and radiolabelled as above under orange Light 2. Cells were harvested at 6500 × *g* for 10 min and resuspended in 10% glycerol, 50 mM Hepes-NaOH, pH 7.6 (this and all subsequent buffers contained 1 mM PMSF and benzamidine as protease inhibitors) prior to disruption by passage twice through a French pressure cell at 1000 psi. Triton X-100 was added to the lysate to 0.1% w/v and the mixture stirred at room temperature for 20 min. After clarification by centrifugation

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at 15000 × *g* for 20 min the lysate was applied to sucrose gradients containing 10–40% sucrose in 50 mM Hepes-NaOH, pH 7.6, with 0.01% Triton X-100. Gradients were centrifuged at 100000 × *g* for 18 h at 4°C, after which a fraction containing approximately 15% sucrose was found to be enriched in the 13 kDa protein. The protein was purified from this fraction by gel filtration on a 2 × 60 cm column of Sephacryl-200 SF (Pharmacia) with 25 mM histidine-HCl, pH 6.2, containing 0.01% Triton X-100 as eluting buffer (flow rate 15 ml·h^{-1}) followed by chromatofocusing in the pH range 6.2–3.8 on a 1 × 25 cm column of PBE94 (Pharmacia) developed with 200 ml Polybuffer (Pharmacia), pH 3.8, containing 0.01% Triton X-100. Fractions containing radiolabeled 13 kDa protein were pooled and concentrated by dialysis against polyethylene glycol (M_r 6000) and then reapplied to the two-stage chromatography process. Radiolabelled fractions resulting from the final chromatofocusing procedure, and found to contain a single protein, were pooled and lyophilized. Residual detergent and Polybuffer were removed by gel filtration through Sephadex LH-60 with formic acid/ethanol (2:1) as eluent [10]. The protein was recovered by lyophilisation in the presence of SDS and subjected to automated N-terminal solid-phase sequencing [11].

Alignment and comparison of sequence data was performed using the Sooty and Sweep programme of the Leeds/Birkbeck ISIS data resource [12].

SDS-PAGE was performed on 12.5–22.5% gels [13] and subsequent autoradiography was with Amersham Hyperfilm MP with intensifying screens at −85°C.

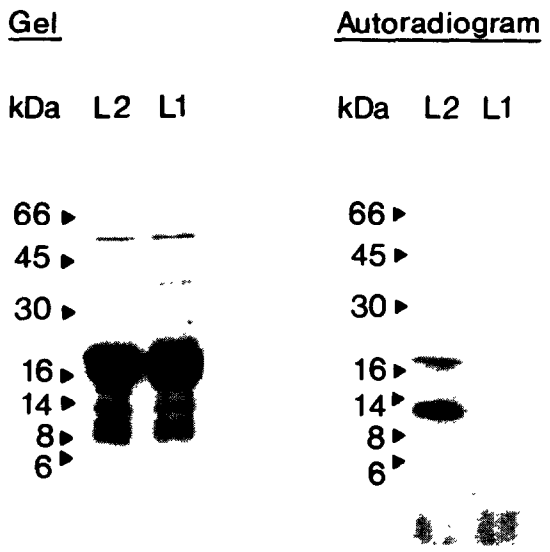

Fig. 1. SDS-PAGE analysis of whole cell polypeptides from *Synechococcus* 6301 (AN112) after incubation with [^{32}P]orthophosphate. L1 and L2 indicate SDS-PAGE tracks of sample derived from cells incubated under Light 1 and Light 2, respectively. Gel, stained gel; Autoradiogram, ^{32}P-labelling of the same gel. Positions and M_r of markers are indicated.

3. RESULTS

Fig. 1 shows comparative levels of protein phosphorylation achieved by *Synechococcus* 6301 cells incubated under Light 2 or Light 1 in the presence of [^{32}P]orthophosphate. Incubation for 30 min under Light 2 gives greatly increased labelling of both the 18.5 and 13 kDa proteins, as previously observed for cells growing under Light 2 [7]. The effects of illumination conditions identical to those used in Fig. 1 on redistribution of absorbed excitation energy between the pigment beds of PS I and PS II are shown in Fig. 2. It is seen that fluorescence emission at 77 K from PS I (at 720 nm) relative to that from PS II (at 685–695 nm) is increased by preillumination with Light 2. In contrast, preillumination with Light 1 causes a decrease in this F720/F685 ratio. These effects indicate altered excitation energy distribution between PS I and PS II [14–17].

Sucrose density gradient centrifugation of whole cell lysate yielded a fraction enriched in both the 18.5 and 13 kDa proteins. Although apparently a soluble protein, recovery of the 13 kDa protein was improved by treatment of the cell lysate with detergent and by inclusion of detergent in the chromatography buffers. Fractionation on Sephacryl-200 removed higher M_r contaminants from the 13 kDa protein, including the 18.5 kDa species (result not shown) as well as providing complete equilibration with the starting buffer for the chromatofocusing procedure. After loading, the chromatofocusing column was washed exhaustively with histidine buffer to remove unbound proteins and developed to pH 3.8, resulting in the elution of radiolabelled protein in the pH range 5.0–4.5. The radiolabelled fractions, containing a mixture of proteins including the 13 kDa protein, were resubjected (after concentration against polyethylene glycol) to the two-stage chromatography procedure. Fig. 3A shows the elution profile of the final chromatofocusing step.

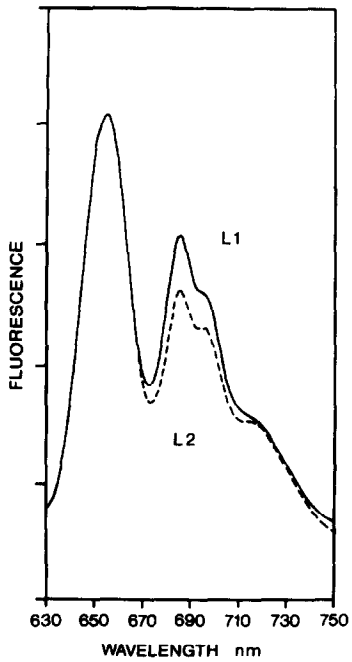

Fig. 2. Fluorescence emission spectra of *Synechococcus* 6301 (AN112) cells at 77 K. Excitation wavelength 600 nm. L1 and L2 indicate cells preilluminated as in Fig. 1. Spectra are normalised to phycocyanin emission at 650 nm.

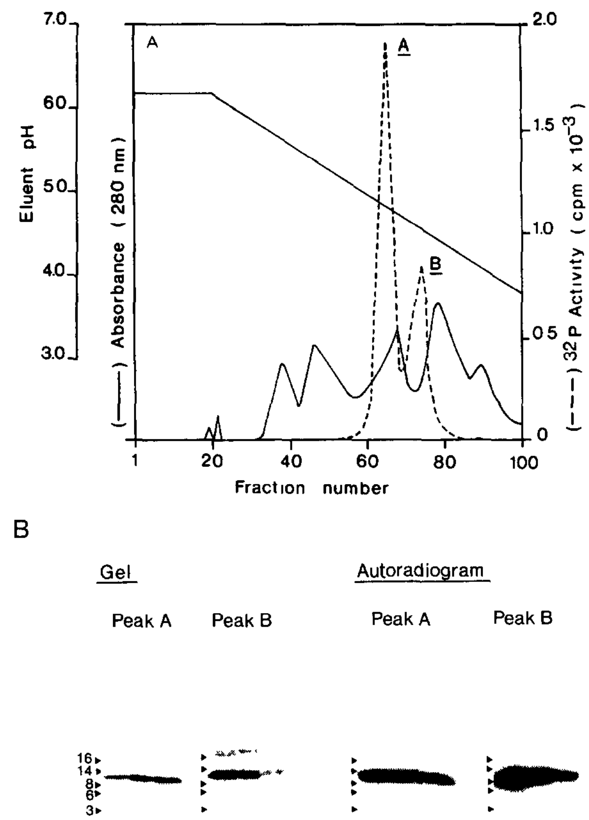

Fig. 3. (A) Elution profile of the final chromatofocusing stage in the purification of the *Synechococcus* 6301 (AN112) 13 kDa protein. Solid line, absorbance at 280 nm; broken line, radioactivity detected by Czerenkov counting. The pH gradient established during the separation is also indicated on the figure. 'Peaks' A and B represent major ^{32}P activity eluting from the column. (B) SDS-PAGE analysis of proteins contained within fractions eluting from the chromatofocusing column in peaks A and B (fractions 64–66 for Peak A; fracations 73–76 for Peak B). Gel, stained gels; Autoradiogram, ^{32}P activity in the same gels. Positions and M_r of markers are indicated.

Two peaks of radioactivity were obtained, eluting at pH 4.9 (Peak A) and pH 4.7 (Peak B), respectively. SDS-PAGE analysis and subsequent autoradiography (Fig. 3B) show Peak A to contain only the purified 13 kDa protein. In addition to several contaminants, Peak B also contains 13 kDa protein which appears to have a greater specific activity than that in Peak A. This may represent the existence of two (or more) pools of the protein with differing phosphate/protein stoichiometries (greater in Peak B), a possibility which could account for the acid shift of the isoelectric point for Peak B.

The Polybuffer and Triton X-100 used in chromatofocusing are non-dialysable and may interfere with protein coupling prior to solid-phase sequencing. Fortunately, they can be adequately removed by gel filtration in non-aqueous solvent systems such as formic acid/ethanol. Fig. 4 shows the sequence of 30 residues obtained from protein extracted from Peak A

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MKXIEAIIRPFKLDEVKIALVNAGIVGMTV  Synechococcus 6301
MKKIDAIIKPFKLDDVRERLAEVGITGMTV  E. coli [18]
MKKIEAIIKPFKLDEVRSP-SGVGLQGITV  R. leguminosarum [19]

MKXIEAIIRPFKLDEVKIALVNAGIVGMTV  Synechococcus 6301
## #*###*#####*#*  # * ## ####  E. coli [18]
## #####*#######* *    #* #*##  R. leguminosarum [19]
```

Fig. 4. The sequence of 30 amino acid residues obtained for the purified 13 kDa protein from *Synechococcus* 6301 (AN112), together with the two sequences of greatest similarity from the OWL database. Amino acid identity is represented by # and a conservative substitution by *.

in Fig. 3 together with the sequences of greatest similarity from the OWL sequence database [12]. All the sequences are aligned from their N-termini. The closest match was obtained between the cyanobacterial 13 kDa protein and the P_{II} protein encoded by the glnB gene of *E. coli* [18]. The two proteins show 63% identity. The *E. coli* P_{II} protein has 102 amino acid residues and a mass of 11.6 kDa. The second closest match for amino acid identity with the cyanobacterial 13 kDa protein is that deduced from the glnB gene from *Rhizobium leguminosarum* [19]. This shows 60% identity with the 13 kDa protein, has 111 residues and a mass of 12.1 kDa. GlnB genes from *Bradyrhizobium japonicum* [20] and *Klebsiella pneumoniae* [21] have also been characterised.

It is possible that the ^{32}P-labelling of the 13 kDa cyanobacterial P_{II}-like protein (Figs 1 and 3) results from uridylylation of tyrosine, as in *E. coli* [18]. Uridylylation of *Synechococcus* P_{II} is consistent with our observation that the ^{32}P-label is removed by phosphodiesterase treatment (results not shown).

4. DISCUSSION

In *E. coli*, P_{II} is uridylylated in response to a decrease in the ratio of glutamine to 2-ketoglutarate in the cell [22], whereupon it activates deadenylylation of glutamine synthetase. It also activates transcription of the gene for glutamine synthetase, glnA, by removal of the unmodified P_{II} that is required as a cofactor for the protein phosphatase activity of a combined kinase-phosphatase activity termed NR_{II} or NtrB. The substrate of NR_{II} therefore becomes phosphorylated. When phosphorylated, this substrate, termed NR_I or NtrC, activates transcription of several *E. coli* genes, including glnA. Phosphorylation of NR_I is likely to directly regulate binding of RNA polymerase to promoters, since NR_I shows the helix-turn-helix motif that is characteristic of DNA-binding proteins and also binds the $sigma^{54}$ RNA polymerase [18,22].

The existence of a glnB-like gene in cyanobacteria suggests the existence in photosynthetic organisms of a redox-linked post-translational and transcriptional control of protein function analogous to the glutamine-

linked regulation of glutamine synthetase found in *E. coli* but absent from the nitrogen-fixing, heterocyst-forming cyanobacterium *Anabaena* 7120 [23]. The redox state of plastoquinone acts as a sensor of imbalance in excitation of the two photosystems in photosynthesis [1,7] and it may control changes in gene expression that give rise to altered photosystem stoichiometry [24,25]. The results in Fig. 1 indicate redox control of cyanobacterial P_{II} modification at the level of plastoquinone.

From the results described here, the possibility arises that post-translational modification during light-state transitions [4] and photosynthetic control of gene expression share common components and that both respond to environmental changes via perturbation of the redox poise of the photosynthetic electron transport chain. Such a two-component regulatory system for photosynthesis could involve components with structural features known to be conserved throughout a wide range of other biological processes [26].

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