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 1Department 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 Pi,, the glnB gene product of *E. coli*. In *E. coli*, the Pi 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, 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.

2. MATERIALS AND METHODS

Cells of *Synechococcus* 6301 (mutant AN112 [9]) were grown to late log phase as in [7] and radiolabelled with [32P]orthophosphate by 10-fold concentration into fresh, phosphate-free growth medium prior to addition of the [32P]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...
at 15000 x 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 x 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 x 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 x 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 (M₂ 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.

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 [³²P]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 M₁ 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. 1. SDS-PAGE analysis of whole cell polypeptides from Synechococcus 6301 (AN112) after incubation with [³²P]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, ³²P-labelling of the same gel. Positions and M₁ of markers are indicated.](image)

![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.](image)
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 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 PI$_2$ protein encoded by the glnB gene of *E. coli* [18]. The two proteins show 63% identity. The *E. coli* PI$_2$ 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 PI$_2$-like protein (Figs 1 and 3) results from uridylylation of tyrosine, as in *E. coli* [18]. Uridylylation of *Synechococcus* PI$_2$ is consistent with our observation that the $^{32}$P-label is removed by phosphodiesterase treatment (results not shown).

4. DISCUSSION

In *E. coli*, PI$_2$ 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 PI$_2$ that is required as a cofactor for the protein phosphatase activity of a combined kinase-phosphatase activity termed NR$_2$ or NtrB. The substrate of NR$_2$ therefore becomes phosphorylated. When phosphorylated, this substrate, termed NK$_1$ or NtrC, activates transcription of several *E. coli* genes, including glnA. Phosphorylation of NR$_1$ is likely to directly regulate binding of RNA polymerase to promoters, since NR$_4$ shows the helix-turn-helix motif that is characteristic of DNA-binding proteins and also binds the sigma$^{34}$ 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 PII 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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**REFERENCES**