

CHARACTERISATION AND PURIFICATION OF POLYPEPTIDES UNDERGOING LIGHT-DEPENDENT PHOSPHORYLATION IN THE CYANOBACTERIUM SYNECHOCOCCUS 6301.

MICHAEL HARRISON AND JOHN F ALLEN
Department of Pure and Applied Biology, University of Leeds, Leeds LS2 9JT, England

1. INTRODUCTION

Chloroplasts and cyanobacteria contain thylakoids which differ greatly in their architecture and composition. Both are, however, able to perform state 1-state 2 transitions, an adaptation mechanism controlling relative distribution of excitation energy between the two photosystems, which permits the optimisation of photosynthetic quantum yield under incident light of varying spectral quality (1,2). In chloroplasts transition to state 2 is induced by phosphorylation of the chlorophyll a/b-binding protein, (LHCII), the mobile antenna of photosystem II, by a kinase the activity of which is controlled by the redox state of plastoquinone (3,4). Phosphorylation induces a decoupling of LHCII from PSII, effecting a decrease in absorbance cross-section for PSII (5,6).

The subject of state transitions in cyanobacteria is controversial (see (7)) but the mechanism is apparently under redox control in a way analogous to that in chloroplasts (8). The role of protein phosphorylation in state transitions in phycobilisome-containing organisms is unclear. Here we report conditions under which three species of polypeptide of M_r 18.5, 15 and 13kDa are phosphorylated in the cyanobacterium Synechococcus 6301.

2. METHODS

Culture and *in vivo* labelling of Synechococcus 6301 (mutant AN112) were performed as in (9) under light regimes specific for excitation of photosystem II and photosystem I defined by filters described in detail in (10). *In vitro* phosphorylation of thylakoids with [³²P]ATP was performed as in (11), (with modifications) under conditions described in the legend to Figure (1).

3. RESULTS AND DISCUSSION

Figure (1) shows the autoradiogram subsequent to SDS-PAGE analysis of Synechococcus 6301 thylakoid proteins incubated with [³²P]-ATP under various conditions (described in the figure legend). From Figure (1) it is

clear that two polypeptides of M_r 18.5kDa and 15kDa are phosphorylated in vitro in a light-dependent fashion in Synechococcus 6301 thylakoids and that this reaction is inhibited by DCMU as described in (11). In addition, both are phosphorylated in the presence of duroquinol in the dark, conditions which would be expected to give slow reduction of the plastoquinone pool. Light incubation in the presence of duroquinone or methyl viologen, which could be expected to give oxidation of plastoquinone, induces essentially no phosphorylation of the 15kDa polypeptide, although labelling of the 18.5 kDa polypeptide, a putative phycobilisome component (9), remains. Presence of duroquinol or methyl viologen in vivo in cyanobacteria has been shown to induce state 2 and state 1 respectively (Mullineaux, CW and Allen, JF, in press).

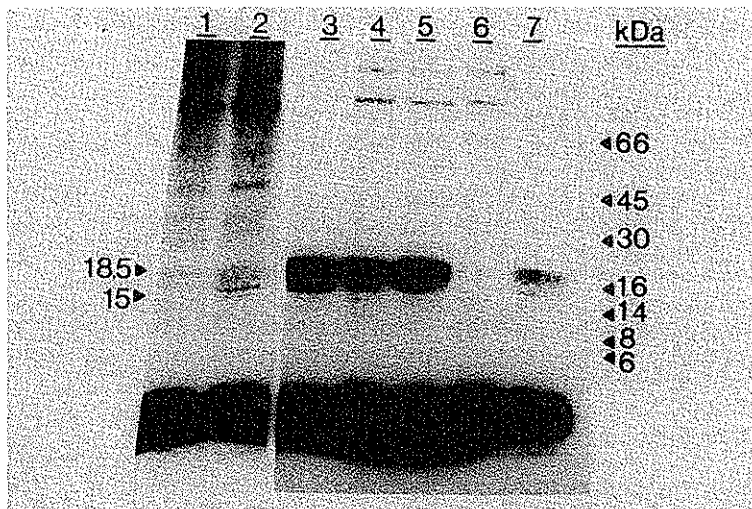


Figure (1) Incubation of Synechococcus 6301 (AN112) thylakoids with [³²P]-ATP. Washed thylakoids were incubated under various conditions, subjected to SDS-PAGE and the resulting gel was autoradiographed. The autoradiogram is shown in the figure. In the interests of brevity the gel is not shown, but tracks showed identical loading (3ug Chl a equivalent thylakoids). Positions of molecular mass markers are shown (kDa). Incubation conditions were: Track (1) Light + 20uM DCMU (2) Light (3) Pretreatment in the light with 20uM DCMU, then light + 0.5mM duroquinol (4) light + 50uM methyl viologen (5) light + 0.5mM duroquinone (6) Dark (7) Dark + 0.5mM duroquinol

We have also observed differential phosphorylation in vivo of both the 18.5kDa polypeptide and another, 13kDa, polypeptide in cells adapted to light regimes which have been found to induce state 1 or state 2 as short term adaptations and also, as longer term adaptations, photosystem stoichiometry changes (10,12). Figure (2) shows SDS-PAGE analysis of polypeptides derived from cells incubated for 30 minutes under light specific for excitation of the phycobilisome, and therefore for PSII, (emission maximum 590nm) or light specific for excitation of PSI (emission maximum 700nm) in the presence of [³²-P] orthophosphate. Autoradiography of the same gel shows labelling of the 18.5 and 13kDa polypeptides almost exclusively under light 2 illumination, conditions known to give state 2 in the short term and to increase the PSI/PSII ratio as a long term adaptation (10,12).

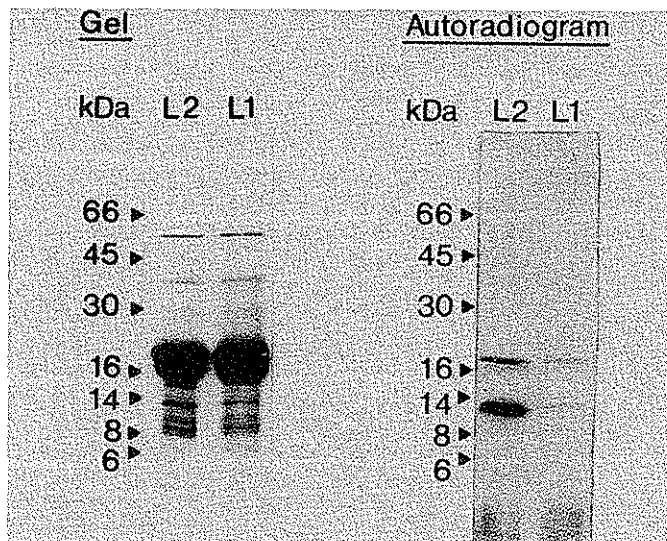


Figure (2) SDS-PAGE analysis of Synechococcus 6301 polypeptides derived from cells labelled in vivo with [³²P] orthophosphate and incubated under PSII light (L2) or PSI light (L1). Left hand figure shows gel of total cell polypeptides. Right hand shows autoradiogram of the same gel.

We have purified and sequenced the 13kDa polypeptide and determined that it has 63% identity with the P_{II} protein of E.coli (13), a protein which is uridylylated as part of a mechanism regulating both the activity of glutamine synthetase and the transcription of its gene,

glnA (14). Details of purification, sequencing and sequence analysis will be published elsewhere (Harrison, MA, Keen, JN, Findlay, JBC and Allen, JF, submitted).

Of the three polypeptides undergoing light-dependent phosphorylation, it is apparent that the 15kDa thylakoid polypeptide at least is substrate for redox-controlled phosphorylation reaction broadly analogous to that for LHCII in chloroplasts. This protein is therefore a likely component for any protein phosphorylation model for the mechanism of state transitions in cyanobacteria.

The intriguing possibility also arises that a PII-type protein in cyanobacteria is a component of a mechanism integrating efficiency of photosynthetic electron transport with nitrogen assimilation, or may even have an effect on transcription of photosynthetic genes, inducing alterations in photosystem stoichiometry as a long-term response to the imbalance in relative turnover of the two photosystems caused by changing spectral quality of incident light (15).

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