THE 18.5 kDa PHOSPHOPROTEIN OF THE CYANOBACTERIUM *SYNECHOCOCCUS* 6301 : A COMPONENT OF THE PHYCOBILISOME

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1. INTRODUCTION

Energy migration between pigment-protein complexes in higher plants is regulated by protein phosphorylation, thus allowing them to adapt to changes in the wavelength of the actinic light. The activity of the protein kinase is regulated by the redox state of the plastoquinone pool (1-5).

Several mechanisms for the control of the distribution of absorbed excitation energy in the cyanobacteria have been proposed; Biggins and co-workers suggest that the state 1 transition is driven by a conformational change that is induced by "Δ", a difference in the charge distribution between PS I and PS II (6). Satoh and Fork suggest that cyclic electron flow around PS I is the controlling factor (7,8). Work in this laboratory (9-13) suggests that the mechanism in the cyanobacteria involves redox-controlled protein phosphorylation and is in this respect similar to that in higher plants.

We have previously reported light-dependent phosphorylation of several polypeptides in the cyanobacterium *Synechococcus* 6301 both *in vivo* and *in vitro* (9,10). One of these polypeptides, of M_r ~ 18.5 kDa, which was isolated from the soluble fraction of whole cells, was tentatively identified as a component of the phycobilisome core (9). Here we show that this polypeptide is a component of the phycobilisome which is phosphorylated in state 2 but not in state 1 and discuss its possible regulatory function.

2. MATERIALS AND METHODS

Synechococcus 6301 (UTEX 625) was grown photoautotrophically in medium C of Kratz and Myers (14) but with the orthophosphate concentration reduced to 0.44 mM. Whole cells were labelled with ^{32}P by growing in low-Pi medium C, containing 50 μCi,ml^{-1} (^{32}P)-orthophosphate, for 3 days, at 35^{o}C, under warm fluorescent lights at about 10 W m^{-2}. The cells were incubated in the dark at 35^{o}C overnight (16 hours) then adapted to state 1 or state 2 by incubation in light 1 or light 2 for 20 minutes at room temperature. Lights 1 and 2 were each provided by two Volpi quartz-halogen 250 W stabilised light sources (CUEL, Kenilworth, UK.) connected to flexible fibre-optic light guides (Schoelly GMBH, FGR). Light 1 was defined by Corning 5-60 filters, and light 2 by a combination of a 657.4 nm short-pass with a 558.2 nm long-pass interference filter.

Intact phycobilisomes were isolated by the method of Grossman and Brand (15), except that, after the initial suspension in 1 M phosphate buffer, the cells were incubated for a further 10 minutes in light 1 or 2 as appropriate. For SDS-PAGE the samples were resuspended by heating at

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90°C for 10 minutes in sample buffer containing 50 mM dithiothreitol, 50 mM Na_2CO_3, 7.5% (v/v) glycerol and 2% (w/v) SDS. They were then run on SDS-PAGE using a 10-30% gradient with 5% stacking gel. The gels were stained with Coomassie blue except where otherwise stated. Phosphorylated polypeptides were detected by autoradiography using Kodak X-OMAT AR film. For 77K fluorescence measurements 10µl of cell suspension, in medium C, or 10µl of the phycobilisome suspension, in 1 M phosphate buffer pH 7.5, was mixed with 90µl of 50% (v/v) glycerol in a capillary tube and quickly frozen in liquid nitrogen.

Fluorescence spectra were recorded using a Perkin-Elmer LS-5 luminescence spectrometer. Chemicals were from BDH or Sigma and were Analar or equivalent. Radioisotope was from New England Nuclear.

3. RESULTS

Fig. 1 shows 77 K fluorescence emission spectra of whole cells incubated in light 2 and light 1. An increase in F720 and decrease in F695 and F685 relative to the phycocyanin peak F650 is seen in cells incubated in light 2 as compared to cells incubated in light 1. This confirms that the light incubation has driven the cells into states 2 and 1 respectively.

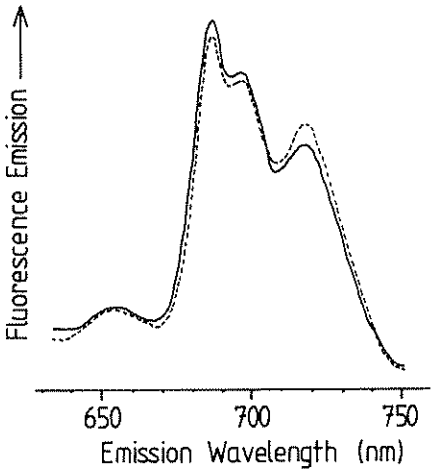

Figure 1.
77 K fluorescence emission spectra of *Synechococcus* cells incubated in light 1 (solid line) and light 2 (broken line). Excitation wavelength 435 nm, slit width 10 nm. Emission slit width 5 nm.

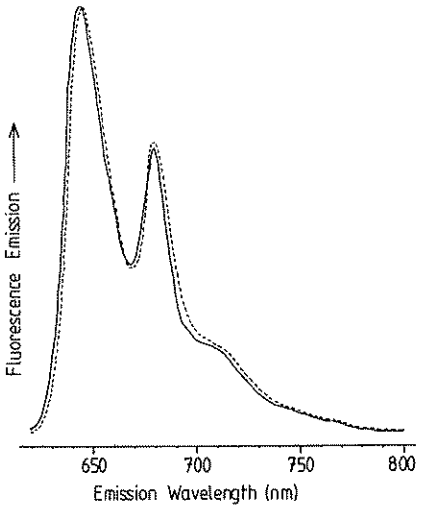

Figure 2.
77 K fluorescence emission spectra of isolated phycobilisomes from *Synechococcus* cells incubated in light 1 (solid line) and light 2 (broken line). Excitation wavelength 580 nm, slit width 2.5 nm. Emission slit width 2.5 nm.

Fig. 2 shows 77 K fluorescence emission spectra of isolated phycobilisomes from cells incubated in light 1 and light 2. There is no significant difference between the two spectra.

Figure 3.
(a) SDS-PAGE gel of isolated phycobilisomes from *Synechococcus* cells incubated in lights 1 and 2. Tracks (i)-(iv) are Coomassie stained and tracks (v) and (vi) are unstained. Tracks (i) and (ii) are molecular weight standards; the relative molecular masses are shown alongside track (i). Tracks (iii) and (v) are phycobilisomes from cells incubated in light 2 (L2) and tracks (iv) and (vi) are phycobilisomes from cells incubated in light 1 (L1).
(b) Autoradiograph of tracks (iii)-(vi) of the gel in (a).

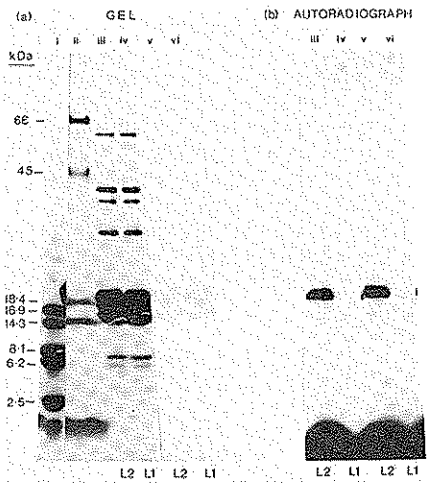

Fig. 3a, shows the SDS-PAGE gel of the isolated phycobilisomes. The Coomassie stained tracks (i-iv) show that the phycobilisomes contain 12 polypeptides ranging from about 10-60 kDa but in the range 15-20 kDa the polypeptides appear blue prior to staining (tracks v and vi); these are the polypeptides which contain the covalently bound phycocyanobilins. Fig. 3b shows the autoradiograph of the gel in Fig. 3a. There is phosphorylation of only one polypeptide of M_r ~18.5 kDa as judged by comparison of electrophoretic mobility with molecular weight markers. The (^{32}P)-incorporation is very much greater in cells incubated in light 2 rather than in light 1. A similar increase in (^{32}P)-incorporation into this phycobilisome polypeptide was seen in cells which were incubated in light as compared to those incubated in the dark (results not shown).

4. DISCUSSION

We have previously suggested (9) that the 18.5 kDa phosphoprotein of *Synechococcus* 6301 is a phycobilisome component. The results presented in this paper strongly support this hypothesis since a phosphoprotein, of similar molecular weight, whose phosphorylation is also light-dependent, may be isolated from a phycobilisome preparation (fig. 3) The result provides support both for our model for the control of light-energy distribution in phycobilisome-containing organisms (9) and for the general model linking protein phosphorylation with co-operatively of light-harvesting systems in photosynthetic organisms proposed in (16-18). We propose that phosphorylation of the 18.5 kDa phycobilisome component and of a 15 kDa membrane component (9,10) leads to their mutual electrostatic repulsion with the consequent redistribution of the phycobilisomes favouring transfer of excitation energy to PS I. Since

the 18.5 kDa protein is phosphorylated to a greater extent in light 2 than in light 1, this would provide a mechanism for optimising the efficiency of light energy utilization similar to that operating in higher plants (1-5) and would provide a molecular mechanism for the state transitions which have been observed in cyanobacteria (19).

The 77K spectra (fig. 1) of whole cells show an increase in fluorescence emission at 720nm and a decrease at 695nm and 685nm relative to the phycocyanin emission at 650nm in light 2-adapted cells as compared to light 1-adapted cells. This confirms that conditions for phosphorylation give rise to a 77K fluorescence emission spectrum characteristic of a transition to state 2. The 77K spectra (fig. 2) of isolated phycobilisomes show no significant difference between phosphorylated and unphosphorylated phycobilisomes. These data support the hypothesis that phosphorylation of the phycobilisome alters excitation energy transfer from the phycobilisome to the membrane-bound pigment-protein complexes but does not affect energy transfer within the phycobilisome.

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