PHOSPHORYLATION OF MEMBRANE PROTEINS

IN CONTROL OF EXCITATION ENERGY TRANSFER

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INTRODUCTION

In chloroplasts of green plants a broad function for phosphorylation of light-harvesting polypeptides is relatively well characterised while the mechanism by which phosphorylation exerts its effect is mostly a matter for speculation. The state of knowledge for the photosynthetic purple bacteria and cyanobacteria is much less satisfactory, and for the green photosynthetic bacteria it is non-existent.

All photosynthetic prokaryotes have quite different light-harvesting polypeptides from those of chloroplasts, and their structure and function cannot therefore be controlled in precisely the same way. Nevertheless, cyanobacteria show light-dependent phosphorylation of polypeptides that can be associated by circumstantial evidence with the light-harvesting phycobilisome and with photosystem II, and purple non-sulphur bacteria show light-dependent phosphorylation of low-molecular-weight chromatophore membrane polypeptides. In both groups phosphorylation can be demonstrated both in vivo and in vitro, and correlates with functional changes observed by chlorophyll fluorescence spectroscopy or chlorophyll fluorescence induction kinetics.

Here we report on recent data concerning light-induced phosphorylation specifically of cyanobacterial polypeptides, purple bacteria having been covered recently elsewhere[1]. In particular, we have now obtained a partial amino acid sequence of a cyanobacterial 13 kDa protein previously suggested to be a light-harvesting protein. Details of the purification technique and of the sequencing protocol will be published elsewhere (M A Harrison, J N Keen, J B C Findlay and J F Allen, submitted). Here we describe this sequence and discuss the implications of our identification of this protein for control of excitation energy transfer at a transcriptional as well as a post-translational level.

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PROTEIN PHOSPHORYLATION IN LIGHT-STATE TRANSITIONS

The cyanobacterial thylakoid membrane differs from that of the chloroplast in that LHC II, the principal antenna complex of photosystem II in chloroplasts, is functionally replaced in cyanobacteria by the phycobilisome. The phycobilisome is a macromolecular assembly of water-soluble polypeptides covalently bound to phycobilin chromophores[2].

Chloroplasts and cyanobacteria nevertheless show functional similarities in the way in which they can adapt the light-harvesting capacity of each photosystem in response to the spectral quality of incident light[3,4]. Light preferentially absorbed by photosystem II (light 2) gives rise to a redistribution of excitation energy in favour of photosystem I, while light preferentially absorbed by photosystem I (light 1) gives rise to a redistribution of excitation energy in favour of photosystem II. The states induced by light 1 and light 2 are termed state 1 and state 2 respectively, and the general phenomenon of redistribution between the two states is known as state 1-state 2 transitions. The effect of each transition is to maintain a high quantum yield of photosynthesis despite an change in the spectral composition of incident light. Without the state transition phenomenon, such a change would favour one or other photosystem to the detriment of the balanced utilization of excitation energy that depends on the series connection of the two photosystems for electron transfer[5,6].

The mechanism of state 1-state 2 transitions in chloroplasts involves phosphorylation of LHC II by a protein kinase whose activity is regulated by the redox state of plastoquinone or of another electron transport intermediate also located betwenn the two photosystems[7,8]. Incident light favouring excitation of photosystem II causes reduction of plastoquinone and hence activation of the kinase that catalyzes phosphorylation of LHC II. This results in dissociation of LHC II from photosystem II and in the attendant changes characteristic of the transition to state 2, including increased relative fluorescence yield at 735 nm at liquid nitrogen temperature that suggests reassociation of phospho-LHC II with photosystem I. Incident light favouring photosystem I causes oxidation of plastoquinone and inactivation of the LHC II kinase. Dephosphorylation of LHC II is then catalyzed by a phosphatase assumed to be continually active, and the state 1 transition follows as the LHC II reassociates with PS II.

Initial experiments on protein phosphorylation in cyanobacteria and red algae showed only light-independent effects[9,10]. Subsequent work, however, showed phosphorylation of polypeptides of molecular weight 18.5 kDa, 15 kDa and 13 kDa in <u>Synechococcus</u> 6301 under conditions shown to correspond to state 2[11]. The 18.5 kDa phosphoprotein is water-soluble and co-purifies with the phycobilisome[12]. The 15 kDa polypeptide is either an integral membrane protein or is bound to the thylakoid membrane[11]. The 15 kDa polypeptide is the principal

species labelled in vitro with gamma(^{32}P)-ATP[13]. The 13 kDa phosphoprotein can be found to occur in both soluble and membrane phases, but is lost from the latter by washing. The 18.5 kDa soluble phosphoprotein was proposed as a phycobilisome component and the 15 kDa membrane phosphoprotein as a component of photosystem II[11], proposals explicitly devised to explain their imagined functions in controlling excitation energy transfer from the phycobilisome to PS II[14]. These proposals have not yet been subjected to the crucial test of purification to the level where amino acid sequencing can be performed, though work on this is currently underway in our laboratory. We have however obtained such results for the 13 kDa protein.

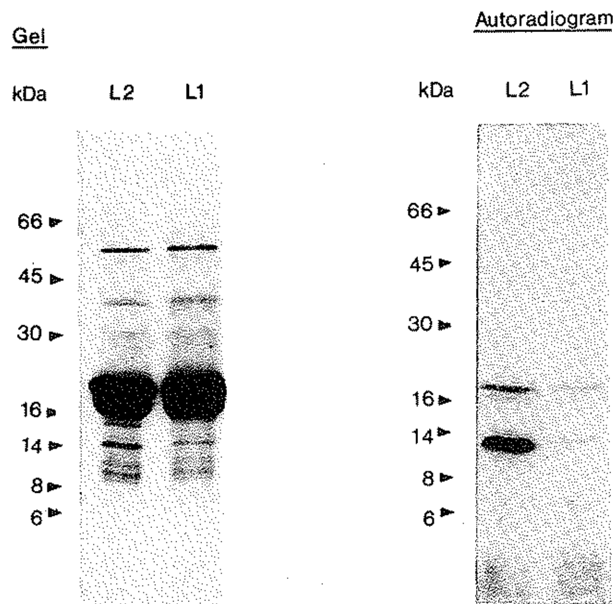

Fig. 1. Whole cell labelling of polypeptides from the cyanobacterium Synechococcus 6301 (mutant AN112) with ^{32}P-orthophosphate. L1 indicates SDS-PAGE track of sample from cells incubated for 30 minutes in light 1, i.e. photosystem I-absorbed light defined by a red filter transmitting light of wavelength greater than 640 nm (50% transmittance)[21]. L2 indicates SDS-PAGE track of sample from cells incubated for 30 minutes in light 2, i.e. photosystem II-absorbed light defined by an orange filter transmitting light between 560 nm and 620 nm (50% transmittance)[21]. "Gel" shows bands stained with Coomassie Brilliant Blue, "Autoradiogram" shows ^{32}P-labelling of the same gel, with molecular weights of standards in kDa as indicated.

THE 13 kDa PROTEIN OF <u>SYNECHOCOCCUS</u> 6301 AN112

Fig. 1 shows effects of 30 minutes' incubation under light absorbed preferentially by photosystem I and photosystem II on ^{32}P-labelling of proteins in vivo in the AN112 mutant[10] of Synechococcus 6301. In the cyanobacteria it is possible to define rigorously wavelength bands specific to photosystems I and II because of the distinctive phycobilin light-harvesting system of photosystem II with an absorption band complementary to that of the chlorophyll light-harvesting system of photosystem I. Fig. 1 shows ^{32}P-labelling of polypeptides running at apparent relative molecular masses of 18.5 kDa and 13 kDa.

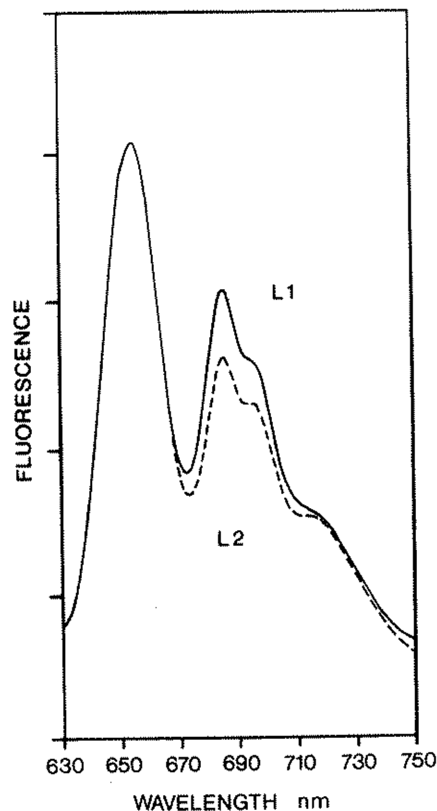

Fig. 2. Fluorescence emission spectra of cells of Synechococcus 6301 (mutant AN112) at 77 K, at 10 microg ml^{-1} chlorophyll concentration with excitation wavelength 600 nm, using a Perkin-Elmer LS5 fluorescence spectrometer. L1 and L2 indicate cells pre-illuminated as in Fig. 1., showing a change characteristic of that between light-state 1 and light-state 2[4,11,21]. Spectra were normalized to the phycocyanin emission maximum at 650 nm.

The effects of the illumination conditions of Fig. 1 on distribution of absorbed excitation energy between the pigment beds of photosystems I and II were confirmed by fluorescence emission spectroscopy, as shown in Fig. 2. Fluorescence emission from photosystem I (at 720 nm) relative to that from photosystem II (at 685 to 695 nm) is increased by illumination, prior to freezing, with light absorbed by photosystem II. In contrast, pre-illumination with light absorbed by photosystem I causes a decrease in low-temperature fluorescence emission from photosystem I relative to that from photosystem II. These effects indicate altered excitation energy distribution between photosystem I and photosystem II, a phenomenon resulting from imbalance in electron transport rates between the two photosystems.

We have developed a procedure for purification of the 13 kDa protein. The procedure gave a single band as detected by staining with Coomassie Brilliant Blue. The ^{32}P-labelled and purified cyanobacterial 13 kDa protein was subjected to acid hydrolysis and the hydrolysate to high-voltage paper electrophoresis. The radioactivity migrated with the tyrosine standard, indicating tyrosine as the the labelled amino acid in the modified 13 kDa polypeptide seen in Fig. 1.

The purified 13 kDa protein was subjected to solid-phase N-terminal sequencing as described. The sequence of 30 amino acids obtained is as follows, together with the sequence of greatest similarity from the OWL protein sequence database of the Leeds-Birkbeck ISIS integrated sequence/integrated structure data resource[15].

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MKXIEAIIRPFKLDEVKIALVNAGIVGMTV   Synechococcus 13 kDa
MKKIDAIIKPFKLDDVRERLAEVGITGMTV   E.coli PII
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With reference to the Synechococcus sequence, an amino acid identity is represented by # and a conservative substitution by * in the E. coli sequence in the following comparison.

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MKXIEAIIRPFKLDEVKIALVNAGIVGMTV   Synechococcus 13 kDa
## #*###*#####*#*  # * ## ####   E.coli PII
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It is seen that the closest match was obtained between the cyanobacterial 13 kDa protein and the P_{II} protein encoded by the glnB gene of E. coli[16], the two proteins having 63% of amino acids in common at each position. The second closest match for amino acid sequence identity with the 13 kDa cyanobacterial protein was for another protein related to E. coli P_{II} whose sequence has been deduced from the sequence of a gene from Rhizobium leguminosarum (not shown). This showed 60% of amino acids identical to those of the 13 kDa protein.

PROTEIN MODIFICATION IN TRANSCRIPTIONAL REGULATION

It is likely that the ^{32}P-labelling of the 13 kDa cyanobacterial P_{II} protein (Figs. 1) results from uridylylation of tyrosine, as in E. coli[17]. In E. coli the P_{II} protein is uridylylated in response to a decrease in ratio of glutamine to 2-ketoglutarate in the cell[17]. The uridylylated form of P_{II} promotes activation of the enzyme glutamine synthetase by deadenylylation. Uridylylation of P_{II} also results in activation of transcription of the glnA structural gene for glutamine synthetase. This activation of transcription results from a removal of the unmodified P_{II} that is required as a cofactor for the protein phosphatase activity of a combined kinase-phosphatase termed NR_{II}. The substrate of NR_{II} therefore becomes phosphorylated as a result of uridylylation of P_{II}. The substrate of NR_{II} is an activator of transcription of several E. coli genes, including glnA, and it activates transcription in its phosphorylated form. This transcriptional activator is the protein NR_I. Phosphorylation of NR_I is likely to regulate directly binding of RNA polymerase at promoters, since the NR_I protein itself shows the helix-turn-helix motif that is characteristic of DNA-binding proteins and also binds the $sigma^{54}$ RNA polymerase[17].

Our discovery of the P_{II} protein in a cyanobacterium suggests the existence in photosynthetic organisms of a combined cascade control of enzyme activity and transcription analogous to that involved in regulation of ammonia assimilation in E. coli. Control of modification of P_{II} by photosynthetic light-harvesting and electron transport (Figs. 1 and 2.) may indicate a coupling between the photochemical reactions of photosynthesis and assimilatory nitrogen metabolism.

TRANSCRIPTIONAL CONTROL OF EXCITATION ENERGY TRANSFER

Light-modified transcriptional regulators such as P_{II} may contribute to feedback control of the synthesis and assembly of the photosynthetic apparatus. For example, in both cyanobacteria[18] and eukaryotic organisms[19] alteration of the stoichiometry of photosystems I and II occurs, with the effect of maintaining equal rates of light utilisation in a process analogous to the short-term redistribution of light-harvesting complexes during state 1-state 2 transitions. The redox state of the electron carrier plastoquinone acts as a sensor of imbalance in excitation of the two photosystems[7] and it has been suggested that changes in plastoquinone redox state initiate changes in gene expression that give rise to altered photosystem stoichiometry[20,21]. Redox control of P_{II} modification at the level of a photosynthetic electron transport component situated between photosystems I and II is indicated by the results in Fig. 1. The possibility therefore arises that short-term light-state transitions[3-7] and photosynthetic control of gene expression share common components and respond to the environmental changes via the same trigger represented by perturbation of redox poise.

Control of light-harvesting phycobiliprotein stoichiometry by complementary chromatic adaptation[22] could also involve redox control of gene expression by such a route.

A feedback control by which components of the photosynthetic apparatus control their own synthesis and assembly may provide an alternative to specific photoreceptors such as phytochrome, whose primary role might then be in non-photosynthetic tissue otherwise incapable of responding to environmental light.

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