Correlation of membrane protein phosphorylation with excitation energy distribution in the cyanobacterium *Synechococcus* 6301

John F. Allen*, Christine E. Sanders and Nigel G. Holmes

Department of Plant Sciences, University of Leeds, Leeds LS2 9JT, England

Received 30 September 1985

Synechococcus cells grown on [^{32}P]orthophosphate exhibit light-dependent phosphorylation of polypeptides at 18.5 kDa (soluble fraction) and 15 kDa (membrane fraction). The 15 kDa polypeptide is also phosphorylated in the light in isolated *Synechococcus* thylakoids incubated with [γ-^{32}P]ATP. 77 K fluorescence emission spectra of both cells and thylakoids show increased photosystem I emission and decreased photosystem II emission under conditions required for protein phosphorylation. We propose that membrane protein phosphorylation regulates distribution of absorbed excitation energy between the two photosystems in *Synechococcus* and other phycobilisome-containing organisms, and that lateral heterogeneity in thylakoid organization is not a necessary condition for protein phosphorylation-dependent adaptations to changing wavelength of light.

Photosynthesis *Cyanobacteria* *Light harvesting* *State 1-state 2 transition* *Protein phosphorylation* *Excitation transfer*

1. INTRODUCTION

In green plants light harvesting is accomplished by intrinsic chlorophyll-protein complexes heterogeneously distributed between appressed and non-appressed regions of thylakoid membrane, and control of distribution of absorbed excitation energy is achieved by phosphorylation of light-harvesting polypeptides and their consequent redistribution between membrane regions specifically enriched with photosystems (PS) I and II [1–9]. In cyanobacteria and red algae light harvesting is accomplished largely by extrinsic phycobiliprotein complexes called phycobilisomes which are thought to be distributed in the same manner as the photosystems, i.e., homogeneously throughout thylakoid membranes that are uniformly unappressed [10–14]. Cyanobacteria and red algae are nevertheless known to exhibit physiological changes in distribution of absorbed excitation energy, demonstrated experimentally as state 1-state 2 transitions [15–19]. The mechanism of these changes is unknown, but it is widely assumed not to involve protein phosphorylation [18,19,20–25] since the model devised for green plants depends on thylakoid lateral heterogeneity. Here we report results consistent with a model which we propose for protein phosphorylation in light-state transitions of phycobilisome-containing organisms.

2. EXPERIMENTAL

Synechococcus 6301 (*Anacystis nidulans*) (UTEX 625) was grown photoautotrophically in medium C of Kratz and Myers [26] but with the orthophosphate concentration reduced to 0.44 mM. Cells were grown to mid-log phase and harvested by centrifugation at 15000 × *g*. Spheroplasts were prepared by a method similar to that in [27]: cells were resuspended in 20% (w/v) sucrose, 10 mM EDTA, 10 mM sodium ascorbate, 10 mM Hepes

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

(pH 7.5), to the original volume of culture, lysozyme was added at 1 $mg \cdot ml^{-1}$ and the cells incubated for 90 min at 35°C. Spheroplasts were centrifuged at 15000 × *g* at 2–4°C and lysed by resuspending the pellet in the original volume of 1 mM Hepes (pH 7.0). An equal volume of solution, containing 10 mM $MgCl_2$, 9 mM Hepes (pH 7.0), and 10 μg/ml RNase and DNase, was added and the suspension stirred for 10 min. The membranes were centrifuged as above then resuspended in 10 mM $MgCl_2$, 15% (v/v) glycerol, 25 mM Hepes (pH 7.5) to a chlorophyll *a* concentration of 1 $mg \cdot ml^{-1}$. Chlorophyll was determined as in [28].

Whole cells were labelled with ^{32}P by growing in low-P_i medium C containing 50 $\mu Ci \cdot ml^{-1}$ [^{32}P]orthophosphate for 3 days. Dark samples were taken from cells grown in the dark for the final 15 h. Using 1 ml samples of culture, membranes were prepared as above (membrane fraction). The soluble fraction was the supernatant formed by centrifugation of the spheroplast lysate. Proteins were precipitated by the addition of 5% trichloroacetic acid. Samples were maintained throughout the fractionation procedure, until trichloroacetic acid precipitation, in the light or dark as appropriate. The protein precipitate was pelleted by centrifugation, then the pellet was resuspended in 200 μl buffer: 1 mM $MgCl_2$, 1 mM $ZnCl_2$, 10 mM Hepes (pH 7.0), containing 1 unit inorganic pyrophosphatase, and incubated overnight (16 h) at 30°C to remove polyphosphates. The pellet was then washed with 80% acetone at −20°C and left for 6 h. The pellet was dried under a stream of nitrogen, resuspended in 100 μl sample buffer, containing 50 mM dithiothreitol, 50 mM Na_2CO_3, 7.5% (v/v) glycerol, 2% (w/v) SDS, by heating to 90°C for 10 min, then treated with RNase (100 $\mu g \cdot ml^{-1}$) overnight at 30°C [29]. Samples were run on SDS-PAGE using a 10–30% gradient with 5% stacking gel. The gels were stained with Coomassie blue. Phosphorylated polypeptides were detected by autoradiography using Kodak X-OMAT S or X-OMAT AR film and autoradiographs were scanned with a Joyce-Loebl Chromoscan 3. For in vitro labelling with [γ-^{32}P]ATP, the membrane suspension was diluted to 20 $\mu g \cdot ml^{-1}$ chlorophyll *a* with 25% (v/v) glycerol, 10 mM $MgCl_2$, 50 mM Hepes (pH 7.5). In some experiments the Mg^{2+} in the membrane suspension buffer and the diluting buffer was replaced with Ca^{2+} but this was found not to affect the phosphorylation of the polypeptides of interest. Using 1 ml samples, the membranes were dark-adapted for 30 min, ATP at 100 $\mu Ci \cdot \mu mol^{-1}$ was added to 0.2 mM and the samples were incubated in the light or dark for 10 min. The reaction was stopped and the membranes precipitated with 5% trichloroacetic acid. The samples were treated as for whole cells omitting the pyrophosphatase and RNase treatments.

For 77 K fluorescence measurements the membranes were diluted to 5 $\mu g \cdot ml^{-1}$ chlorophyll *a* with 50% (v/v) glycerol, 10 mM $MgCl_2$, 50 mM Hepes (pH 7.5). Samples were dark-adapted for 30 min, 0.2 mM ATP added and 60 μl of sample transferred to a capillary tube. The tubes were incubated for 10 min in the light or dark then quickly frozen in liquid N_2. The whole cells were dark-adapted for 30 min then 10 μl of cell culture mixed with 50 μl of 50% (v/v) glycerol in a capillary tube which was then treated as for membranes. Fluorescence spectra were recorded using a Perkin-Elmer LS-5. Enzymes were from Sigma and radioisotopes from Amersham.

3. RESULTS

Fig.1a shows densitometric scans of autoradiographs of 2 tracks from a single SDS-PAGE slab gel of protein samples from *Synechococcus* cells grown on [^{32}P]orthophosphate. Cells incubated in the light (90 min) show ^{32}P labelling of 4 bands in the membrane fraction, corresponding to Coomassie blue-stained polypeptides of 20, 18.5, 15 and 13 kDa as judged by comparison of electrophoretic mobility with molecular mass markers. The adjacent track loaded with sample from dark-incubated cells shows greatly decreased labelling of the 18.5 and 15 kDa polypeptides, with the 20 and 13 kDa bands relatively unchanged. This result indicates that phosphorylation of the 18.5 and 15 kDa polypeptides is light-dependent in vivo. The 18.5 kDa band is seen as the most conspicuously labelled polypeptide in the soluble fraction of ^{32}P-grown *Synechococcus* cells, and labelling is also seen there to be light-dependent [25]. The 15 kDa band is absent from the soluble fraction [25].

Fig.1b shows corresponding scans of autoradiographs of 2 tracks of samples of

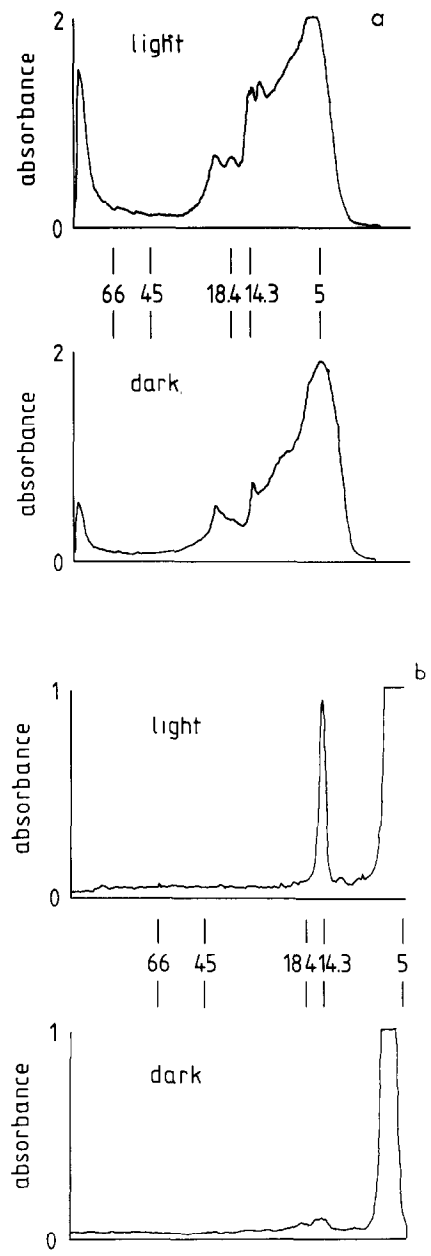

Fig.1. Densitometric scans of autoradiographs of SDS-PAGE slab gel tracks of labelled *Synechococcus* polypeptides, prepared as in section 2. (a) Membrane fraction of whole cells grown on $^{32}P_i$. (b) Thylakoids labelled in vitro by incubation with [γ-^{32}P]ATP (0.2 mM). Numbers show position of molecular mass markers (in kDa) in adjacent tracks.

Synechococcus thylakoids, isolated by osmotic lysis of spheroplasts, and incubated (10 min) with [γ-^{32}P]ATP (0.2 mM) in light or dark. A major labelled band is seen at 15 kDa, with a minor band at 13 kDa. Labelling in both cases is light-dependent, with little labelling of the 15 kDa band and no labelling of the 13 kDa band being seen in the dark control. The conclusion from fig.1 is that membrane-bound polypeptides of 15 and 13 kDa are phosphorylated in the light in vivo and in vitro and that a soluble or weakly membrane-bound polypeptide of 18.5 kDa is phosphorylated in the light in vivo.

Fig.2a shows 77 K fluorescence emission spectra

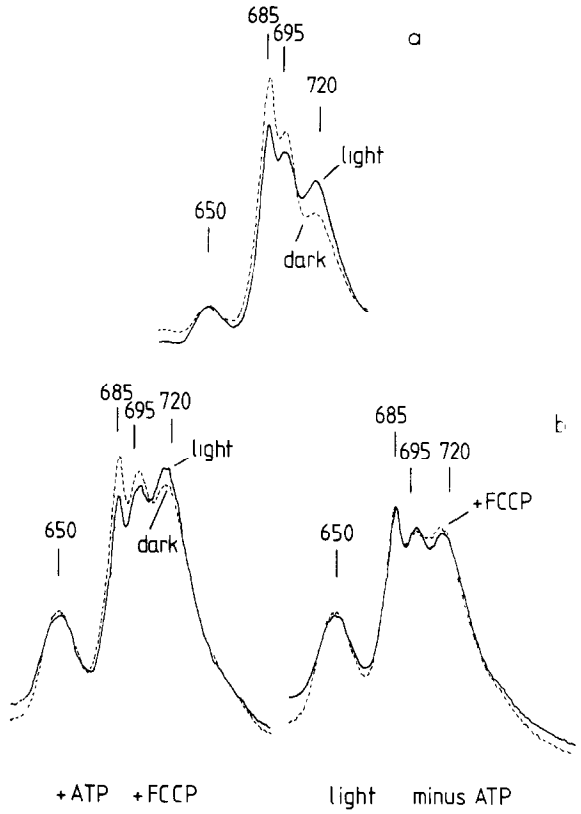

Fig.2. 77 K fluorescence emission spectra of *Synechococcus* cells and thylakoids, prepared as in section 2. Excitation wavelength 435 nm, 15 nm slit width. Emission slit width 2.5 nm. (a) Whole cells incubated in light or dark prior to freezing. (b) Thylakoids incubated with ATP (0.2 mM) and FCCP (10 μM) in light or dark prior to freezing. (c) Whole cells incubated in the light without ATP, with and without FCCP, prior to freezing.

of unlabelled *Synechococcus* cells incubated for 30 min in the dark and then incubated for a further 10 min in light or dark prior to freezing. A light-dependent increase in F_{720} and decrease in F_{695} and F_{685} is seen relative to the phycocyanin peak F_{650}. The dark spectrum corresponds to state 1 and the light spectrum to state 2.

Fig.2b shows 77 K fluorescence emission spectra of *Synechococcus* thylakoids prepared as for the labelling experiment (fig.1b) and incubated in light or dark in the presence of ATP and FCCP prior to freezing. A light-dependent increase in F_{720} and decrease in F_{695} and F_{685} is seen, indicating that state 2 can be induced by light in uncoupled thylakoids in the presence of ATP. Fig.2c shows equivalent 77 K fluorescence emission spectra obtained from thylakoids pre-incubated in the absence of ATP. In this case the relative peak heights characteristic of state 1 are maintained even after illumination, with omission of the uncoupler FCCP having little effect except possibly to decrease F_{720} to a small extent.

The data of fig.2 together indicate that under appropriate conditions a light-induced state 2 transition occurs both in *Synechococcus* cells and in isolated *Synechococcus* thylakoids provided ATP is present, while fig.1 demonstrates that light-induced protein phosphorylation occurs in both experimental systems under similar conditions.

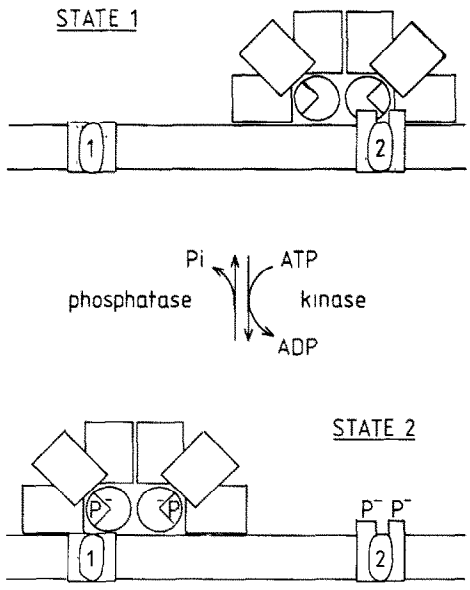

Fig.3. Model for control by protein phosphorylation of excitaion energy transfer in phycobilisome-containing organisms. State 2 is induced by phosphorylation (i) of the 18.5 kDa polypeptide that forms one sector of a disc of the phycobilisome core and (ii) of a surface-exposed segment of a 15 kDa component of the intrinsic antenna complex of PS II (antenna complexes represented as stippled areas). Mutual electrostatic repulsion or conformational dissociation between the phycobilisome and PS II then acts in a direction parallel to the membrane plane, serving to alter the pathway of excitation energy transfer in favour of PS I at the expense of PS II.

4. DISCUSSION

This letter reports light-induced phosphorylation of soluble and membrane polypeptides of the cyanobacterium *Synechococcus* 6301. The conditions for protein phosphorylation also produce changes in 77 K fluorescence emission which indicate altered excitation energy distribution in favour of PS I. The same correlation of protein phosphorylation with transition to state 2 occurs in vitro in *Synechococcus* thylakoids. On this basis we propose the model in fig.3 for state 1-state 2 transitions in cyanobacteria and red algae. The model assumes that the 18.5 kDa phosphoprotein is identical to the 18.3 kDa linker protein of the phycobilisome core [30], and that the 15 kDa membrane phosphoprotein is an antenna chlorophyll-binding protein of PS II. We propose that phosphorylation of these 2 polypeptides causes their mutual electrostatic repulsion or conformation changes causing their dissociation and hence to increased excitation energy transfer to PS I at the expense of PS II.

We conclude that redox-controlled protein phosphorylation is directly responsible for state 1-state 2 transitions in phycobilisome-containing organisms, with electrostatic decoupling of the phycobilisome from PS II acting instead of the 'lateral shuffling' of light-harvesting complex of green plants. Lateral heterogeneity in thylakoid organization would not then be a necessary condition for such an adaptive mechanism, but rather an additional, stabilizing mechanism found in some green plants.

ACKNOWLEDGEMENTS

We thank Dr Hilary Evans for helpful discussion and gifts of cell cultures, and Dr Howard Atkinson for use of the gel scanner. Supported by SERC and Royal Society grants to J.F.A.

REFERENCES

[1] Allen, J.F. (1983) Trends Biochem. Sci. 8, 369–373.
[2] Allen, J.F., Bennett, J., Steinbeck, K.E. and Arntzen, C.J. (1981) Nature 291, 25–29.
[3] Barber, J. (1983) Photobiochem. Photobiophys. 5, 181–190.
[4] Barber, J. (1982) Annu. Rev. Plant Physiol. 33, 261–295.
[5] Bennett, J. (1983) Biochem. J. 212, 1–13.
[6] Howarth, P., Kyle, D.J., Horton, P. and Arntzen, C.J. (1982) Photochem. Photobiol. 36, 743–748.
[7] Horton, P. (1983) FEBS Lett. 152, 47–52.
[8] Horton, P. and Black, M.T. (1980) FEBS Lett. 119, 141–144.
[9] Kyle, D.J., Kuang, T.-Y., Watson, J.L. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 89–96.
[10] Stanier, R.Y. and Cohen-Bazire, G. (1977) Annu. Rev. Microbiol. 31, 225–274.
[11] Gantt, E. (1981) Annu. Rev. Plant Physiol. 32, 327–347.
[12] Glazer, A.N. (1984) Biochim. Biophys. Acta 768, 29–51.
[13] Tandeau de Marsac, N. (1983) Bull. Inst. Pasteur 81, 201–254.
[14] Larkum, A.W.D. and Barrett, J. (1983) Adv. Bot. Res. 10, 1–219.
[15] Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251.
[16] Ried, A. and Reinhardt, B. (1977) Biochim. Biophys. Acta 460, 25–35.
[17] Ley, A.C. and Butler, W.L. (1980) Plant Physiol. 65, 714–722.
[18] Fork, D.C. and Satoh, K. (1983) Photochem. Photobiol. 37, 421–427.
[19] Bruce, D., Biggins, J., Steiner, T. and Thewalt, M. (1985) Biochim. Biophys. Acta 806, 237–246.
[20] Miller, K.R. and Lyon, M.K. (1985) Trends Biochem. Sci. 10, 219–222.
[21] Biggins, J., Campbell, C.L. and Bruce, D. (1984) Biochim. Biophys. Acta 767, 138–144.
[22] Biggins, J. and Bruce, D. (1985) Biochim. Biophys. Acta 806, 230–236.
[24] Satoh, K. and Fork, D.C. (1983) Biochim. Biophys. Acta 722, 190–196.
[25] Sanders, C.E., Holmes, N.G. and Allen, J.F. (1986) Biochem. Soc. Trans., in press.
[26] Kratz, W.A. and Myers, J. (1955) Am. J. Bot. 42, 282–287.
[27] Hellingwerf, K.J., Michels, P.A., Dorpma, J.W. and Komings, W.N. (1960) Eur. J. Biochem. 55, 397–406.
[28] Strain, H.H., Cooper, B.J. and Svec, W.A. (1971) Methods Enzymol. 23, 452–476.
[29] Wang, J.Y.J. and Koshland, D.E. (1978) J. Biol. Chem. 253, 7605–7608.
[30] Glazer, A.N. (1983) Annu. Rev. Biochem. 52, 125–157.