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Effects of divalent cations on 77 K fluorescence emission and on membrane protein phosphorylation in isolated thylakoids of the cyanobacterium *Synechococcus* 6301

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The effects of divalent cations (Mg^{2+}; Ca^{2+}; Zn^{2+}) on the phosphorylation of membrane-bound polypeptides in *Synechococcus* thylakoids were investigated. The effects of divalent cation and ATP concentration on the corresponding changes in 77 K fluorescence emission were also measured. The light-dependent phosphorylation of a 15 kDa polypeptide was shown to be dependent also on the presence of divalent cations, with Mg^{2+} and Ca^{2+} being the most effective. The 15 kDa polypeptide did not become dephosphorylated in the dark. This was shown to be due to a lack of phosphatase activity in this thylakoid membrane preparation. The light-induced changes in 77 K fluorescence emission spectra of *Synechococcus* thylakoids were shown to be independent of divalent cation concentration, to occur in the absence of ATP, and to be irreversible. The results from fluorescence emission spectroscopy are contrary to the conclusions of previous work in our laboratory (Allen, J.F., Sanders, C.E. and Holmes, N.G. (1985) FEBS Lett. 193, 271–275). The results indicate that the changes in fluorescence emission do not arise from a transition to light State 2: they may result instead from photooxidation of chlorophylls of Photosystem II. Since the 77 K fluorescence changes do not report on state transitions, we conclude that their independence of ATP is not inconsistent with a role for reversible protein phosphorylation in state transitions in cyanobacteria.

Introduction

A wide range of photosynthetic organisms, including green plants, red algae and cyanobacteria, regulates the distribution of excitation energy between PS I and PS II in response to changes in actinic light [1–4]. It is widely accepted that in green plants this is achieved by membrane protein phosphorylation where the activity of the protein kinase is regulated by the redox level of the plastoquinone pool [5–7]. For cyanobacteria and red algae other mechanisms have been proposed, involving spill-over from PS II to PS I [8], the charge distribution between PS I and PS II [9] and cyclic electron flow around PS I [10,11]. For a review of these putative mechanisms, see Ref. 12.

Work in our laboratory [13–18] suggests that the mechanism of regulation in the cyanobacteria involves redox-controlled protein phosphorylation

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem; PQ, plastoquinone; F_0, fluorescence level when all PS II traps are open; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; λ_{ex}, excitation wavelength; PAGE, polyacrylamide gel electrophoresis.

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and is, in this respect, similar to that in green plants. We have previously reported the light-dependent phosphorylation, both in vivo and in vitro, of several polypeptides of the cyanobacterium *Synechococcus* 6301 [14] and have correlated phosphorylation with changes in the 77 K fluorescence emission spectra [13,18].

Biggins and Bruce [19] recently reported that similar changes in 77 K fluorescence emission spectra of *Synechococcus* thylakoids can be observed in the absence of ATP and are independent of cation concentration. Biggins and Bruce [19] conclude that the membrane protein phosphorylation is light-independent in vitro.

Here we confirm that the membrane protein phosphorylation is light-dependent in *Synechococcus* thylakoids. We also report the effects of divalent cations both on the in vitro phosphorylation of 15 kDa, membrane-bound polypeptide of *Synechococcus* 6301 and on the associated changes in the 77 K fluorescence emission spectra. The results presented here indicate that the changes in 77 K fluorescence emission are not the result of a State-2 transition and that they are not ATP-dependent. The latter result is contrary to the conclusion from previous results from our laboratory [13]. However, since the fluorescence changes do not report on state transitions, the fact that they are not ATP-dependent does not exclude a role for membrane protein phosphorylation in state transitions in phycobilisome-containing organisms.

Materials and Methods

Synechococcus 6301 (UTEX 625) was grown and thylakoid membranes were isolated from sphaeroplasts as in Ref. 13, except that the final membrane pellet was resuspended in 10 mM $MgCl_2$, 10% (v/v) glycerol, 10 mM Hepes (pH 7.5) to a chlorophyll *a* concentration of 1.5–2 $mg \cdot ml^{-1}$. Chlorophyll was determined as in Ref. 20.

In vitro labelling with [γ-^{32}P]ATP was given in Ref. 13, except that the membrane suspension was diluted with the above resuspension medium in which the $MgCl_2$ was either omitted or replaced with $CaCl_2$ or $ZnCl_2$ as appropriate, and the final concentration of ATP was 0.1 mM at 200 $\mu Ci \cdot \mu mol^{-1}$ specific activity. Where indicated, samples were treated with 200 $\mu g \cdot ml^{-1}$ protease (from *Streptomyces griseus*) at 30 °C for 6 h immediately prior to electrophoresis. 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 Amersham Hyperfilm-MP.

For the time-course of ^{32}P incorporation, 10 ml membrane suspension (at 20 $\mu g \cdot ml^{-1}$ chlorophyll *a*) was stirred in the dark for 15 min. ATP at 200 $\mu Ci \cdot \mu mol^{-1}$ was added to 0.1 mM and a 1 ml sample was withdrawn immediately. The stirred suspension was illuminated with a 60 W tungsten filament light at 30 cm and further 1 ml samples were withdrawn at $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 5, 10 and 15 min after the start of illumination. All the samples were precipitated with 5% trichloroacetic acid immediately after their removal from the reaction vessel. The samples were then treated as above for SDS-PAGE and autoradiography. ^{32}P incorporation was measured as the area under the peak corresponding to the 15 kDa phosphoprotein on a densitometric scan of the autoradiograph. Autoradiographs were scanned with a Joyce-Loebl Chromoscan 3.

To monitor phosphatase activity a time-course of ^{32}P incorporation was measured as above, except that 15 ml of membrane suspension was used with 10 mM $MgCl_2$ present throughout. A sample was taken in the dark and at $\frac{1}{2}$, 1, 2, 3, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$ and 15 min after the start of illumination. The stirred suspension was then put into the dark and a sample was taken after $2\frac{1}{2}$, 5, 10 and 15 min dark incubation. For pre-incubation with ATP, the membrane suspension was stirred in the dark for 15 min. Cold ATP was added to 0.1 mM and the stirred suspension was illuminated (as above) for 15 min. [γ-^{32}P]ATP was then added to 200 $\mu Ci \cdot \mu mol^{-1}$. Samples were withdrawn during 15 min illumination and 15 min dark incubation as above. Samples were treated for SDS-PAGE and autoradiography and the ^{32}P incorporation was estimated as above.

77 K fluorescence measurements were made as in Ref. 13, except that the membrane suspension was diluted with 10% (v/v) glycerol, 10 mM Hepes (pH 7.5), containing 10 mM $MgCl_2$ or $CaCl_2$ as appropriate. All reagents were Analar or equiv-

alent. Enzymes were from Sigma and radioisotopes from New England Nuclear.

Results

Fig. 1a shows four tracks from a single SDS-PAGE gel of protein samples from *Synechococcus* thylakoids, isolated by osmotic lysis of sphaeroplasts and incubated with [γ-^{32}P]ATP in the light or dark in the presence of 10 mM $MgCl_2$, $CaCl_2$, $ZnCl_2$ or without divalent cations. Fig. 1b shows the autoradiograph of the entire gel. A single-labelled band is seen at 15 kDa (judged by comparison with known molecular weights of marker proteins) the phosphorylation of which is light dependent. There is maximum ^{32}P incorporation into this band in the presence of either $MgCl_2$ or $CaCl_2$, less ^{32}P incorporation in the presence of $ZnCl_2$ and virtually none without divalent cations. When the protein samples are treated with protease prior to SDS-PAGE, the Coomassie-stained bands are absent from the gel and the labelled band is absent from the autoradiograph showing that the ^{32}P is bound to a protein. The conclusion from Fig. 1 is that a membrane-bound polypeptide of molecular mass 15 kDa is phosphorylated only in the light and that this phosphorylation is dependent on the presence of

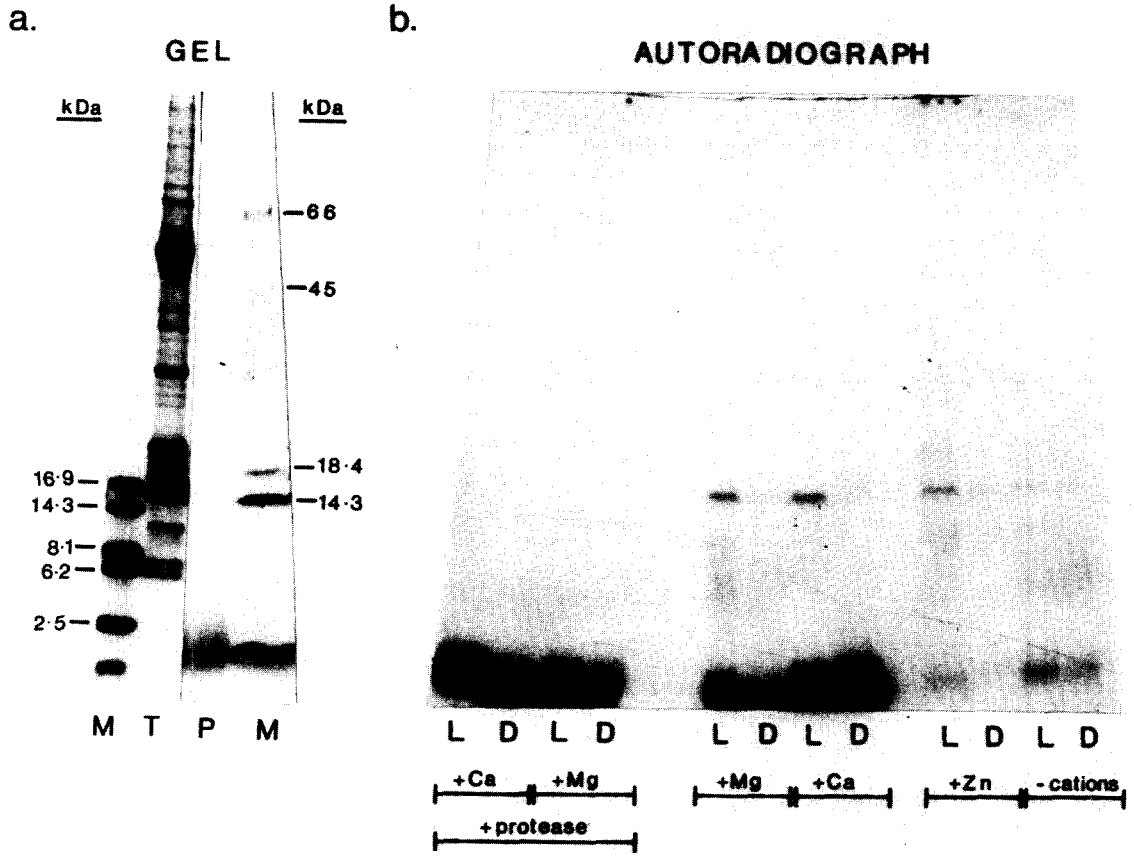

Fig. 1. (a) SDS-PAGE gel of thylakoid membrane proteins. Tracks from left to right are: molecular-weight markers (M); thylakoid membrane proteins (T); thylakoid membrane proteins treated with protease prior to electrophoresis (P); molecular-weight markers (M). The relative molecular mass of the markers in kDa is shown alongside the gel. (b) Autoradiograph of thylakoid membrane proteins run on an SDS-PAGE gel. Samples were incubated in the light (L) or the dark (D), in the presence of 10 mM $MgCl_2$ (+Mg), 10 mM $CaCl_2$ (+Ca), 10 mM $ZnCl_2$ (+Zn) or without divalent cations (−cations). Where indicated, ('+protease'), samples were treated with protease prior to electrophoresis.

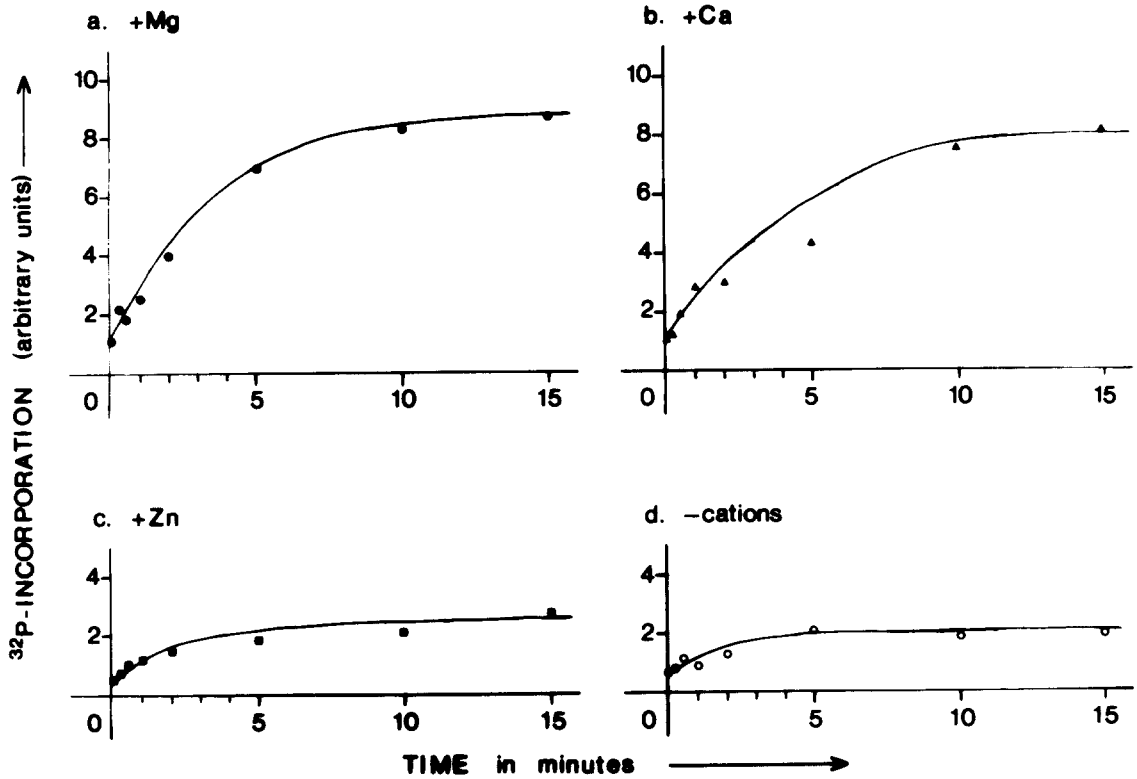

Fig. 2. Time-course of ^{32}P incorporation into the 15 kDa polypeptide. The amount of ^{32}P incorporation was measured by scanning the autoradiograph tracks of samples run on SDS-PAGE and measuring the area under the peak corresponding to the 15 kDa-labelled polypeptide. ^{32}P incorporation was measured in the presence of (a) + 10 mM $MgCl_2$ (●——●), (b) + 10 mM $CaCl_2$ (▲——▲), (c) + 10 mM $ZnCl_2$ (■——■), and (d) without divalent cations (○——○).

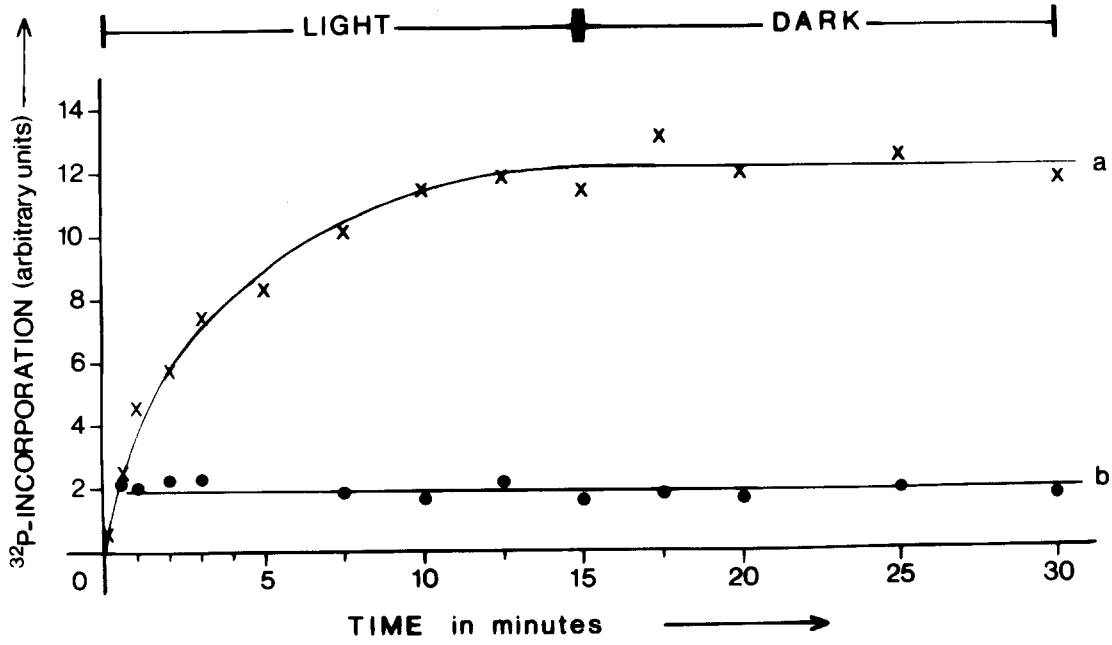

Fig. 3. Time-course of ^{32}P incorporation into the 15 kDa polypeptide during incubation in the light and the dark, in the presence of 10 mM $MgCl_2$. ^{32}P incorporation was measured as in Fig. 2. (a) ×——×, [γ-^{32}P]ATP added to dark-adapted membranes; (b) ●——●, [γ-^{32}P]ATP added to membranes pre-incubated in the light with unlabelled ATP.

divalent cations, Mg^{2+} and Ca^{2+} being the most effective.

Fig. 2 shows a time-course for the ^{32}P incorporation into the 15 kDa polypeptide in the presence of $MgCl_2$, $CaCl_2$, $ZnCl_2$ and without divalent cations. The patterns of ^{32}P incorporation in the presence of $MgCl_2$ and $CaCl_2$ are very similar, the only difference being that the maximum incorporation in the presence of $CaCl_2$ is only 95% that with $MgCl_2$. The maximum ^{32}P incorporation in the presence of $ZnCl_2$ and without divalent cations is 25% and 20%, respectively of the maximum with $MgCl_2$. However, although the total amount of ^{32}P incorporation differs with the different cations present, the half-time for the phosphorylation reaction to reach a steady state remains constant at about 1.5–2 min.

Fig. 3 shows a time-course for ^{32}P incorporation into the 15 kDa polypeptide during 15 min illumination followed by 15 min in the dark. Fig. 3(a) shows ^{32}P incorporation into dark-adapted membranes. As previously (Fig. 2a), on illumination the level of ^{32}P incorporation rises to a steady state reaching the maximum within approx. 10 min. When the light is switched off, however, the level of phosphorylation remains constant, indicating that dephosphorylation does not occur in the dark. Fig. 3(b) shows ^{32}P incorporation into membranes which have been pre-incubated in the light with unlabelled ATP. There is a small initial incorporation of ^{32}P into the 15 kDa polypeptide in the light which remains constant throughout the period of illumination and dark incubation.

Fig. 4 shows 77 K fluorescence emission spectra of *Synechococcus* thylakoids incubated in the presence of $MgCl_2$ in the light or dark prior to freezing. Fig. 4a and b show spectra obtained with 435 nm excitation ($\lambda_{ex} = 435$ nm). It can be seen that samples incubated in the light show an increase in fluorescence emission at 719 nm (F_{719}) relative to fluorescence emission at 697 nm (F_{697}) and a decrease in F_{697} relative to F_{719} compared to

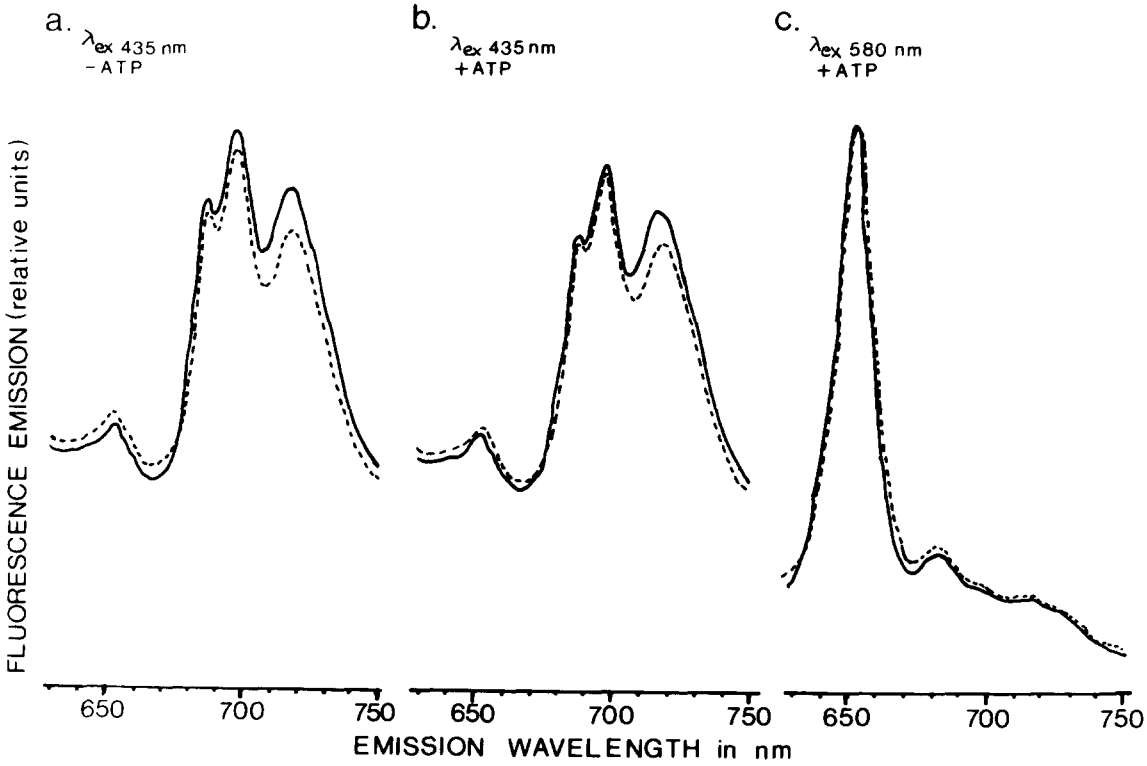

Fig. 4. Fluorescence emission spectra of *Synechococcus* thylakoids, in the presence of 10 mM $MgCl_2$, incubated in the light (solid line) or the dark (broken line). Excitation slit width was 2.5 nm and emission slit width was 5 nm. (a) Excitation at 435 nm ($\lambda_{ex} = 435$ nm); (b) + 0.1 mM ATP, excitation at 435 nm ($\lambda_{ex} = 435$ nm); (c) + 0.1 mM ATP, excitation at 580 nm ($\lambda_{ex} = 580$ nm).

TABLE I

EFFECT OF CATIONS AND ATP ON THE LIGHT-INDUCED CHANGE IN THE 77 K FLUORESCENCE EMISSION OF *SYNECHOCOCCUS* THYLAKOID MEMBRANES

Ratio of 77 K fluorescence emission at 697 nm (F_{697}) to that at 719 nm (F_{719}) for *Synechococcus* thylakoid membranes incubated in medium containing either 10 mM $MgCl_2$ ($+Mg^{2+}$) or 10 mM $CaCl_2$ ($+Ca^{2+}$) or no divalent cations (−cations), and containing 0.1 mM ATP where indicated (+ATP). Samples were incubated in the light (L) or the dark (D) for 10 min prior to freezing. Excitation was at 435 nm, slit width was 2.5 nm, emission slit width was 5 nm. Each value is a mean of four samples with the range given in brackets.

Incubation conditions	Ratio of F_{697}/F_{719}		% Change in F_{697}/F_{719} caused by light incubation
	dark	light	
$+Mg^{2+}$	1.168 (1.10–1.22)	1.068 (0.94–1.13)	−8.6%
$+Mg^{2+}$ +ATP	1.125 (1.08–1.16)	1.048 (0.94–1.11)	−6.8%
$+Ca^{2+}$	1.15 (1.08–1.22)	1.085 (0.99–1.15)	−5.7%
$+Ca^{2+}$ +ATP	1.163 (1.08–1.28)	1.078 (1.03–1.14)	−7.5%
−Cations	1.098 (0.97–1.20)	1.145 (1.01–1.26)	+4.3%
−Cations+ATP	1.15 (1.20–1.30)	1.103 (0.95–1.22)	−4.1%

those incubated in the dark. This change in the ratio F_{697}/F_{719} occurs both in the presence (Fig. 4b) and absence (Fig. 4a) of ATP. Table I shows the change in the ratio F_{697}/F_{719} for thylakoids incubated in the presence of $MgCl_2$, $CaCl_2$ and without divalent cations, with and without ATP. In all cases, except thylakoids incubated in the absence of cations and ATP, there is a decrease in the F_{697}/F_{719} ratio for thylakoids incubated in the light compared to those in the dark. Table II shows the changes in the F_{697}/F_{719} ratio when thylakoids are incubated in the dark for 10 min, followed by 10 min light then returned to the dark for 10 min. As before, on transfer from the dark to the light, there is a decrease in the F_{697}/F_{719} ratio but on returning to the dark there is a further decrease in the F_{697}/F_{719} ratio. The data in Table II relate to thylakoids incubated in the presence of Mg^{2+}, but the same effect was seen with Ca^{2+} and in the absence of divalent cations (results not shown). These data indicate that the light-induced decrease in the F_{697}/F_{719} ratio is not reversible.

Fig. 4c shows fluorescence emission spectra of *Synechococcus* thylakoids with excitation at 580 nm (λ_{ex} = 580 nm). The spectra obtained with 435 nm excitation (Fig. 4a and b) are similar to those obtained with whole cells excited at 435 nm [13,18]. In contrast, spectra of thylakoids excited at 580 nm (Fig. 4c) show only two fluorescence emission maxima at 650 nm and 685 nm. They are different from those obtained with whole cells excited through the phycobilisomes in that the latter show four fluorescence emission maxima at approx. 650 nm, 685 nm, 697 nm and 719 nm [21]. The fluorescence emission maxima at 697 nm and 719 nm originate in PS II and PS I, respectively [22]. Their

TABLE II

IRREVERSIBILITY OF LIGHT-INDUCED CHANGES IN THE 77 K FLUORESCENCE EMISSION OF *SYNECHOCOCCUS* THYLAKOID MEMBRANES

Ratio of 77 K fluorescence emission at 697 nm (F_{697}) to that at 719 nm (F_{719}) for *Synechococcus* thylakoid membranes incubated in medium, containing 10 mM $MgCl_2$ and containing 0.1 mM ATP where indicated (+ATP). Samples were incubated in dark for 10 min (D), transferred to the light for 10 min (DL) then returned to the dark for 10 min (DLD) prior to freezing. Excitation was at 435 nm, slit width 2.5 nm, emission slit width 5 nm. Each value is a mean of four samples with the range of values given in brackets.

Incubation conditions	Ratio of F_{696}/F_{719}	% Change in F_{697}/F_{719}
$+MgCl_2$		
D	1.175 (1.11–1.25)	–
DL	1.12 (1.07–1.19)	−4.7%
DLD	1.05 (0.99–1.17)	−6.3%
$+MgCl_2$ +ATP		
D	1.185 (1.15–1.25)	–
DL	1.118 (1.05–1.17)	−6.0%
DLD	1.06 (0.93–1.13)	−5.8%

absence from thylakoid membranes indicates that the 580 nm light, which is absorbed by the phycobilisomes, is not transferred to the PS II or PS I reaction centres, and hence that the phycobilisomes are not functionally connected to PS II. The spectra in Fig. 4c appear similar to those obtained with isolated intact phycobilisomes [17], where the large 650 nm peak originates from the phycocyanin and allophycocyanin and the 685 nm peak from the terminal emitter polypeptide which anchors the phycobilisome to the membrane.

Discussion

When *Synechococcus* cells undergo state transitions they show a characteristic change in their 77 K fluorescence emission spectra: on transition from State 1 to State 2 there is a decrease in the F_{697}/F_{719} ratio. However, the light-induced decrease in F_{697}/F_{719} ratio in *Synechococcus* membranes reported here differs from that seen in whole cells in three respects. Firstly, the change in F_{697}/F_{719} ratio in membranes is not reversible within 10 min, whereas state transitions are readily reversible. The half-time for the transitions to State 1 and to State 2 are about 15 and 30 s, respectively [16]. Secondly, the change in ratio seen in membranes occurs in the absence of divalent cations, while it has been reported that depleting *Synechococcus* cells of calcium prevents state transitions [23]. Thirdly, the light-induced decrease in F_{697}/F_{719} ratio in membranes is very much smaller than that seen in whole-cell state transitions; typically, there is a decrease in the F_{697}/F_{719} ratio of 20–30% in cells on transition to State 2 [13,18], whereas the decrease in membranes is only 4–8%. We therefore conclude that the decrease in F_{697}/F_{719} ratio seen in this membrane system is not due to a light-induced State-2 transition, and furthermore, since it is seen to occur in the absence of ATP, it cannot be correlated with the light-induced protein phosphorylation.

Similar light-induced changes in the F_{697}/F_{719} ratio in a *Synechococcus* membrane preparation have been reported by Biggins and Bruce [19] who also found that the changes occur in the absence of Mg^{2+} or ATP. Further, they report that these changes: (i) occur in EDTA-washed or gluteraldehyde-fixed preparations; (ii) are unaffected by DCMU; and (iii) can be induced in the dark by potassium ferricyanide. However, in contrast to our findings (Table II), they found that these changes were fully reversible, Biggins and Bruce [19] concluded that the light-induced fluorescence changes are most likely caused by a photooxidation of a pigment associated with PS II rather than by a state transition. This view is consistent with the results presented here.

Further to our earlier observation [13], the results presented here confirm that the phosphorylation of the 15 kDa polypeptide is virtually unchanged when Ca^{2+} replaces Mg^{2+} in the reaction medium. This is not the result of a carry-over of Mg^{2+} from the resuspension medium to the reaction medium (giving a final Mg^{2+} concentration of about 0.1 mM). If this were the case, there would be a similar amount of phosphorylation seen in membranes incubated in '−cations' medium as in membranes incubated with 10 mM $MgCl_2$. Figs. 1 and 2 clearly show that reducing the $MgCl_2$ to 0.1 mM ('−cations' medium) results in an 80% reduction in ^{32}P incorporation into the 15 kDa polypeptide. Neither is Ca^{2+}-dependent phosphorylation the result of a general effect of divalent cations, since replacing Mg^{2+} instead with Zn^{2+} results in a greatly reduced phosphorylation. If Ca^{2+} can replace Mg^{2+} because of a general charge-screening effect, then any divalent cation (e.g., Zn^{2+}) should also be as effective. The reduction in ^{32}P incorporation when Zn^{2+} replaces Mg^{2+} is not the result of an inhibition of the phosphorylation reaction by Zn^{2+}. This effect would only be inhibition if the addition of Zn^{2+}, in the presence of Mg^{2+}, caused a decrease in phosphorylation. The amount of phosphorylation in the presence of Zn^{2+} is slightly higher than that in the '−cations' medium (Fig. 2c and d). As we have explained above, the residual phosphorylation in the '−cations' medium is due to the carry-over of Mg^{2+} from the resuspension medium. If the Zn^{2+} was causing an inhibition of the phosphorylation reaction, we would expect to see less phosphorylation in the presence of Zn^{2+} than in the '−cations' medium.

As previously reported, the phosphorylation is, in all cases, light-dependent. These results are contrary to the findings of Biggins and Bruce [19],

who report that the kinase activity of the membrane preparation is dependent on magnesium, but who concluded that there is essentially the same labelling profile in the light and the dark. Although the conclusion of Biggins and Bruce [19] is that there is not a requirement for light for the phosphorylation of the 15 kDa polypeptide, careful examination of the densitometric scan in Fig. 2 of Biggins and Bruce [19] shows a 30% light-induced stimulation of the phosphorylation relative to the off-set baselines of each scan.

Fig. 3a shows that the 15 kDa polypeptide does not become dephosphorylated in the dark. This is the opposite of what would be expected if the light-dependent phosphorylation in cyanobacterial membranes operated by a system similar to that in higher plant thylakoids. In higher plants, phosphorylation is achieved by competing kinase and phosphatase enzymes catalysing phosphorylation and dephosphorylation reactions, respectively. The activity of the kinase is controlled by the redox level of PQ and the phosphatase has a constant activity [5–7]. In the dark PQ is rapidly oxidised and the kinase is deactivated allowing dephosphorylation. The lack of dephosphorylation in this membrane preparation could be due to the kinase remaining active in the dark or to a lack of phosphatase activity.

In a kinase/phosphatase system, the phosphorylation seen when the kinase is active is the net result of the phosphorylation and dephosphorylation reactions. Although there appears to be a constant level of phosphorylation there is a continuous exchange of phosphate groups between the ATP pool and the phosphorylated polypeptides. If [γ-^{32}P]ATP is added, in the light, to a preparation of thylakoid membranes phosphorylated with unlabelled ATP, then the exchange between the ATP pool and the phosphorylated polypeptide should lead to a fairly rapid incorporation of ^{32}P orthophosphate into the phosphopolypeptide. Fig .3b shows that in this membrane preparation this exchange does not occur. Since we know that the kinase is active under these conditions (Figs. 2 and 3a), we must conclude that the phosphatase activity is absent. It seems unlikely that lack of phosphatase activity is due to the conditions being wrong. The kinase has been shown to operate under these conditions and both the phosphorylation and the dephosphorylation reactions have been shown to operate under the same conditions (except for light) in vivo [13]. It would therefore be unlikely that the conditions for phosphatase activity are greatly different to those for kinase activity. The loss of activity may be due to the loss of the soluble phosphatase during preparation of the membranes or because dephosphorylation in cyanobacterial membranes is achieved by some other means.

Our method of preparation of the thylakoid membranes results in the detachment of the majority of the phycobilisomes from the membranes, and those remaining are not functionally connected to PS II (Fig. 4c). Under these conditions, with the major light-harvesting assembly missing from PS II, it is not surprising that the membranes do not exhibit the large fluorescence changes associated with state transitions in whole cells. Although the data reported here indicate that the changes in 77 K fluorescence emission cannot be attributed to a light-induced State 2 transition, there are still grounds to suppose that state transitions are controlled by protein phosphorylation linked to the redox level of PQ, since the conditions required for phosphorylation are conditions under which PQ would be expected to be reduced. The lack of dephosphorylation under conditions where PQ would be expected to be oxidised is explained by the lack of phosphatase activity in this membrane preparation.

The original model which we proposed for state transitions [13] suggests that the phycobilisome dissociates from PS II in State 2. There is good evidence for this dissociation from fluorescence induction transients where a large decrease in F_0, indicating a decrease in the absorption cross-section of PS II, is seen with phycobilisome-absorbed light, but not with chlorophyll-absorbed light [25]. There is still, therefore, evidence to suggest that the State-2 transition in cyanobacteria involves the decoupling of the phycobilisome from PS II and that this is controlled by reversible protein phosphorylation, a mechanism consistent with the kind of control of photosynthetic unit function envisaged in Ref. 24.

We conclude that the original fluorescence data presented in Ref. 13 did not report on a light-induced State-2 transition in vitro. The absence of

an ATP requirement for these changes [13,19] does not therefore rule out a protein phosphorylation mechanism for the State-2 transition of cyanobacteria in vivo.

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