Biochimica et Biophysica Acta, 1100 (1992) 75–82

BBABIO 43587

Characterization of light-activated reversible phosphorylation of a chlorophyll *a/b* antenna apoprotein in the photosynthetic prokaryote *Prochlorothrix hollandica*

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(Received 24 June 1991)

Key words: Thylakoid protein; Protein phosphorylation; Chlorophyll *a/b* antenna; (*P. hollandica*)

Phosphorylation of thylakoid proteins of 35, 25, 23 and 14 kD was shown to occur in *Prochlorothrix hollandica* both in vivo and in vitro. In vivo: the kinase was active in high light, but not in low light or dark. Next to the 35 kDa protein, which formed the major target, 25 and 23 kDa proteins were phosphorylated. PS II light (650 nm) stimulated kinase activity, which was inactive in PS I light (710 nm). In vitro: the kinase was found to be constantly active, and inhibitors of photosynthetic electron transport had no effect on phosphorylation levels. Prolonged dark incubation before isolation of thylakoid membranes did yield a partially inactivated kinase. Rapid activation could be obtained by both light and addition of the plastoquinol analogue duroquinol. Phosphorylation of the 23 and 25 kDa proteins was enhanced by addition of antimycin A under illuminating conditions and by sodium fluoride. The latter observation suggests that thylakoid proteins may be phosphorylated by the same kinase, but are dephosphorylated at different rates. The 35 kDa phosphoprotein was shown to form a chlorophyll *a/b* antenna copurifying with PS I. Light-regulated reversible phosphorylation of a chlorophyll *a/b* binding protein in *P. hollandica* immunologically related to that of *Prochloron* indicates that a similar mechanism may operate in the latter. The hypothesis was raised that reversible phosphorylation of a 35 kDa apoprotein of the chlorophyll *a/b* antenna results in a change in its orientation to PS I by which it enables/enhances energy transfer to PS II. It is suggested that this antenna is structurally associated with PS I. Lastly, a 33 kDa polypeptide was identified as a chlorophyll *a/b* binding protein which may form an antenna to PS II.

Introduction

State 1 → 2 transitions, the process of energy redistribution between photosystem (PS) I and PS II, are known to occur in higher plants and green algae [1–4], red algae and cryptomonads [5–8] and cyanobacteria [9–12]. The molecular mechanism underlying this phenomenon is not the same for all groups. In higher plants and green algae state 1 → 2 transitions are established by lateral movement of the light-harvesting complex II (LHC II) between the PS II centers in the grana and the stromal PS I centers and they were related to the reversible phosphorylation of the LHC II proteins [13]. It was thought that the thylakoid-bound kinase responsible for phosphorylation in green chloroplasts was controlled by the redox state of the PQ-pool [14]. More recent data indicate that the quinol binding site of the cytochrome b_6/f complex is directly involved in regulation of kinase activity in a number of species [4,15–17].

In phycobilisome-containing organisms like red algae and cyanobacteria it is less clear what molecular mechanism mediates state 1 → 2 transitions. Although the lack of a thylakoid-intrinsic PS II antenna in these organisms seems to exclude the mechanism of lateral movement, reports appeared on the role of protein phosphorylation in regulating energy (re)distribution [11,18,19]. It is now generally believed that state 1 → 2 transitions in cyanobacteria are established through

Abbreviations: Chl, chlorophyll; PS I, Photosystem I; PS II, Photosystem II; PQ, plastoquinone; PQH_2, plastoquinol; cyt b_6/f, cytochrome b_6/f; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; NEM, *N*-ethylmaleimide.

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changes in energy spillover from the PS II Chl *a* antenna to PS I. The faster time-course of state I → 2 transitions in red algae and cyanobacteria led to the exclusion of protein phosphorylation as a molecular mechanism in favor of modulation of redox states of electron carriers and/or localized charge distributions [7,20,21].

Prochlorophytes are another group of photosynthetic prokaryotes apparently related to cyanobacteria [22]. They lack phycobilisomes but possess a Chl *a/b* antenna structurally different from the LHC II in green chloroplasts [23–28]. A 34 kDa Chl *a/b* binding protein in *Prochloron* is phosphorylated in a light-independent way [23] and this pigment-protein complex copurifies with PS I [26]. The permanently phosphorylated antenna may prevent state transitions in vivo [23]. Also in *Prochlorothrix hollandica* most of the Chl *b* is bound to proteins of 30–35 kDa copurifying with PS I complexes [28]. In analogy to *Prochloron* this organism seems to lack some or all of the features thought to be necessary for state 1 → 2 transitions, including a light-harvesting complex discriminating between light 1 and 2. Moreover, phosphorylation of a number of proteins in crude membrane preparations was found to be light-independent [29]. However, state transitions visualized as changes in PS II fluorescence yield were observed upon red/far red transients [30]. The question thus rises about the sensitizing pigment complex, the nature of the system monitoring the balance in PS II and PS I activity, and the molecular mechanism active in adjusting this balance. Here, we have identified a light-controlled kinase activity in vivo located in the thylakoid membrane. In vitro a prolonged dark incubation is needed to partially inactivate the kinase. The kinase is involved in reversible phosphorylation of a subset of the chlorophyll *a/b* antenna proteins, specifically a 35 kDa apoprotein copurifying with PS I.

Materials and Methods

P. hollandica was maintained in batch cultures under light limiting conditions in the nutrient sufficient BG11 medium [31]. Cultures were continuously mixed on an orbital shaker at 22°C with incident illumination of 25 μmol quanta m^{-2} s^{-1} obtained from 2 'warm white' fluorescent tubes placed at 50 cm from the cultures. The pH of the cultures was 8.3 ± 0.3.

Thylakoid membrane preparation

For thylakoid preparation [23] cells were harvested by centrifugation for 5 min at 5000 rev/min, washed in TMN buffer containing 50 mM Tris-HCl (pH = 8.0), 10 mM NaCl, 5 mM $MgCl_2$, 100 mM sorbitol and 1 mM benzamidine, and pelleted as before. Pellets were resuspended in TMN buffer and broken by passing the sample through a French press cell operated at 12500 lb/in^2. The homogenate was then loaded on a cushion of 60% sucrose in TMN buffer and centrifuged for 20 min at 40000 rev/min in a Beckman Ti 50 rotor at 4°C, thus separating thylakoid membranes from cell debris including plasma membranes. The green interphase was collected, washed in TMN buffer and pelleted at 45000 rev/min as described above. Thylakoids obtained in this procedure retained high PS I and PS II activities and chlorophyll *a/b* ratios were similar to those found for whole-cell extracts (8:1).

Phosphorylation studies

In vitro phosphorylation studies were performed according to Gal et al. [4] using thylakoid membranes at a concentration of 9 μg Chl *a* per 100 μl reaction mixture. Inhibitors were added together with the radioactive label and their final concentrations are reported in the figure legends of the results section. Illumination conditions for in vitro phosphorylation were 200 μmol quanta m^{-2} s^{-1} of white light for 10 min unless stated otherwise. The reaction was stopped by transfer to a 10% solution of cold TCA. After 5 min resolubilization in the presence of 3% LDS at 80°C, the membranes were electrophoresed on 10–17% continuous gradient polyacrylamide gels.

For in vivo phosphorylation, log phase cells were washed and resuspended in fresh BG 11 medium [31] containing 1/10 the original phosphate concentration. 125–250 ml cultures were allowed to equilibrate for 2 h at 20 μmol quanta m^{-2} s^{-1} in low phosphate medium containing 1 μCi/ml $H_3^{32}PO_4$ (ICN radiochemicals, 500 mCi/mmol) and subsequently transferred to either dark or 150 μmol quanta m^{-2} s^{-1}. Monochromatic illumination with intensities of 20 μmol quanta m^{-2} s^{-1} was obtained by passing light from a fibre optic light source through double band interference filters with transmission maxima centered around 650 and 710 nm. Whole cells were lysed by Deriphat extraction and prepared for electrophoresis as described above. The reaction was stopped by transfer to ice followed by centrifugation at 5000 × *g*, washed and resuspended to 350 μg chlorophyll *a* ml^{-1} in TMN buffer without sorbitol. To this suspension, Deriphat 160 (Serva) was added dropwise from a 30% solution to give a final concentration of 1%. After homogenization the mixture was incubated for 30 min at 4°C followed by centrifugation at 20000 × *g* for 15 min. The resulting green supernatant was used for electrophoresis on 14% denaturing SDS–polyacrylamide gels after solubilization for 5 min at 70°C in 3% LDS sample buffer. For non-denaturing gel electrophoresis ^{32}P labelled membranes were solubilized in 30% LDS sample buffer at 4°C and electrophoresed on prechilled (4°C) 10% polyacrylamide gels.

Standard methods

Western blotting and immunodecoration were performed essentially as described by Towbin et al. [32], employing the modifications of Smith and Fisher [33]. Chlorophyll was determined from 90% acetone extracts and concentrations were calculated from the equations of Arnon [34]. Proteins were determined by the Lowry procedure [35]. The antibody to the 30 kDa CP 5 Chl *a/b* antenna apoprotein has been described previously [36].

Results

In vitro phosphorylation of P. hollandica thylakoid proteins

To examine whether in isolated thylakoid membranes the pattern of protein phosphorylation resembled that in chloroplasts of higher plants and green algae, we performed, in vitro, phosphorylation studies to detect (a) thylakoid bound protein kinase and phosphatase activities and (b) thylakoid proteins exhibiting reversible phosphorylation. Phosphorylation with [^{32}P]ATP for 15 min yielded mainly a 35 kDa phosphoprotein labelled equally in light and dark (Fig. 1). The amount of ATP incorporated was 1.2 nmol μg^{-1} thylakoid protein under these conditions. In the presence of the phosphatase inhibitor NaF, and independent of light, two more phosphoproteins became visible at 23 and 25 kDa apparent molecular mass, whereas the intensity of label associated with the 35 kDa protein did not change. Since kinase activity may be modulated by changing the redox state of the PQ-pool or the cytochrome b_6/f complex, we performed these experiments in the presence of inhibitors of linear/cyclic electron transport. In vitro phosphorylation in the light was not affected by addition of the quinone analogues DCMU, DBMIB or duroquinol (Fig. 2). NaF had identical effect on the phosphorylation of the 23 and 25 kDa proteins for all inhibitor additions. This suggests that kinase activity in vitro is not regulated by photosynthetic electron transport or that the PQ-pool is continuously reduced. The latter option could be excluded, since fluorescence induction characteristics of isolated thylakoids indicated the presence of an oxidized PQ-pool in dark-adapted thylakoids (Fig. 3), which in principle allows for redox controlled re-

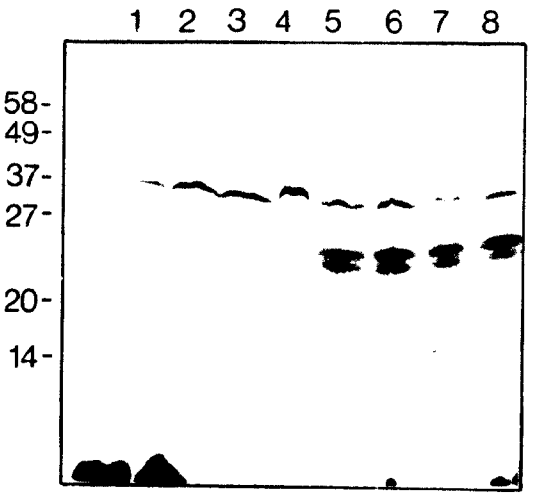

Fig. 2. Autoradiogram of electrophoresed *P. hollandica* thylakoid proteins after in vitro phosphorylation in light in the absence (lanes 1–4) and presence (lanes 5–8) of 10 mM NaF. [1,5] Control, [2,6] + 10 μM DCMU, [3,7] + 10 μM DBMIB, [4,8] + 10 mM duroquinol.

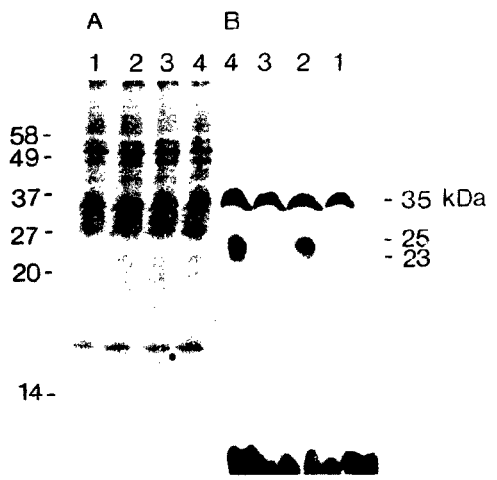

Fig. 1. Coomassie blue gel (left) and autoradiogram (right) of *P. hollandica* thylakoid proteins after in vitro phosphorylation in [1] light, [2] light + 10 r. M NaF, [3] dark and [4] dark + 10 mM NaF.

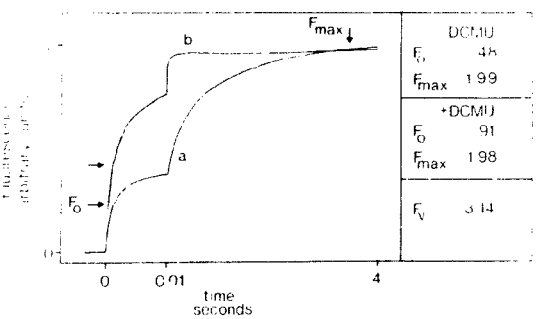

Fig. 3. Fluorescence induction curves for isolated thylakoid membranes of *P. hollandica* in the absence (a) and presence (b) of 10^{-6} M of DCMU. Excitation was 200 μmol quanta m^{-2} s^{-1} of light < 500 nm and emission was determined for wavelengths > 680 nm. F_o was determined during the fast fluorescence rise (0–10 ms) at 2.5 ms after shutter opening, whereas F_{max} was measured in the slow phase of fluorescence rise (0.010–4 s) at 3.75 s. F_v was calculated as $F_{max} - F_o$ normalized to F_{max}.

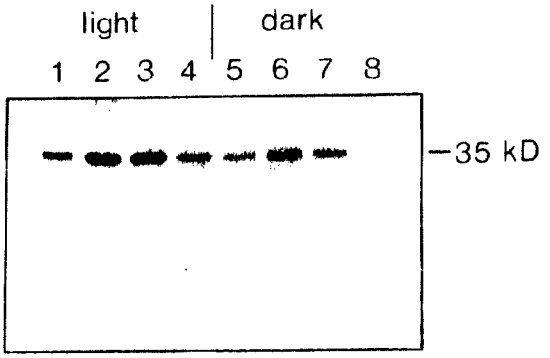

Fig. 4. In vitro phosphorylation of *P. hollandica* thylakoids isolated after a 10 h dark incubation of culture suspension. [1] light control; [2] light + 10 mM NaF; [4] light + 10 mM duroquinol; [4] light + 1 μM DCMU [5,6] as for [1-4] under dark conditions. The 23 and 25 kDa phosphoproteins became visible in the NaF treated samples only after prolonged exposure of dried gels.

versible phosphorylation. Prolonged dark incubation before isolation of thylakoids yielded a largely inactivated kinase (Fig 4). Whereas the kinase was immediately activated in the light, the effect was enhanced by addition of either NaF (inhibiting dephosphorylation) or duroquinol (a PQH_2 analogue). These data suggest the presence of a light/redox-controlled kinase, which is rapidly activated in the light and slowly inactivated in the dark.

Antimycin A, an inhibitor of cyclic electron flow around PS I [37], invoked enhanced phosphorylation of the 23 and 25 kDa proteins in the light (Fig. 5). Simultaneous addition of NaF and antimycin A re-

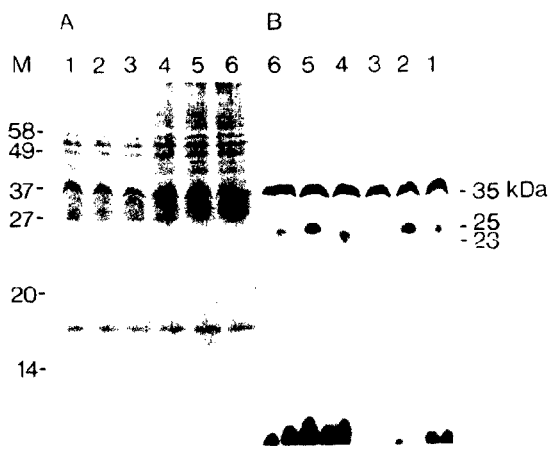

Fig. 5. Coomassie blue gel (left) and autoradiogram of *P. hollandica* thylakoid proteins after in vitro phospho light + 10 μM antimycin A; [2] light + 10 μM antimyc NaF; [3] dark + 10 μM antimycin A; [4] dark + 10 μM 1 10 mM NaF; [5] dark + 10 mM NEM and 10 mM Na ins + 10 mM NEM and 10 mM NaF.

Fig. 6. In vivo phosphorylation of *P. hollandica* thylakoid proteins. [1,2] Cells grown in the presence of 1 μCi $H_3^{32}PO_4$ ml^{-1} in low phosphate medium at 20 μmol quanta m^{-2} s^{-1} (M) for 3 h. [3,4] Same as lanes 1 and 2 except the cultures were shifted to 150 μmol quanta m^{-2} s^{-1} (H) for 3 h. The 23 and 25 kDa phosphoproteins are only visible after longer exposure.

sulted in equally strong phosphorylation. This effect was not observed in the dark, where only NaF was active in enhancing phosphorylation. Addition of NEM did not significantly affect phosphorylation, indicating

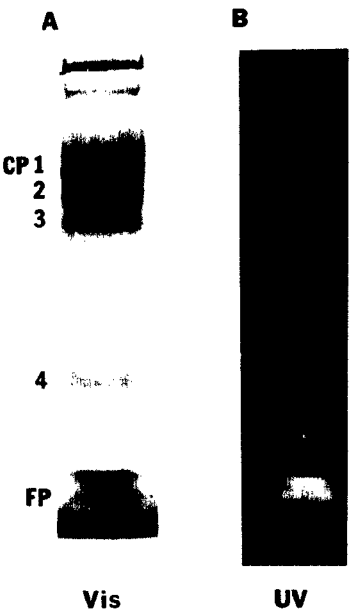

Fig. 7. Chlorophyll protein complexes of *P. hollandica*. Deriphat extracts were electrophoresed under non-denaturing conditions [35]. Chlorophyll-protein complexes were photographed during transillumination with visible (panel A) and long wavelength UV light (panel B). CP2 and CP3 form the major chlorophyll *a*/*b* antenna complexes due to their Chl *a*/*b* ratio of 2.5.

that the kinase is not activated through ~SH bonds. After trypsin digestion of thylakoid membranes the phosphorylated peptide fragments of the 23 and 25 kDa proteins appeared as a diffuse band below 10 kDa (data not shown), suggesting that the phosphorylated protein domains are surface exposed with respect to the membrane. The 35 kDa phosphoprotein was relatively resistant to trypsin treatment, in accordance with its strongly hydrophobic nature which predicts it to be almost completely embedded in the thylakoid membrane [36].

In vivo phosphorylation of P. hollandica cells

Phosphorylation in *P. hollandica* cultures was studied to determine whether the kinase activity phosphorylated the same complement of thylakoid proteins in vivo. Cells incubated at high light showed similar phosphorylation patterns as observed in the in vitro experiments (Fig. 6). Based on the $^{32}P/^{33}P$ ratio of the medium and assuming steady-state labelling of the intracellular phosphate pools, we calculated a minimal rate of phosphorylation of 6.4 pmol ATP/μg total protein per h. An additional 14 kDa phosphoprotein, observed only after prolonged exposure of in vitro labelled thylakoids, was apparent. Medium light (and dark, not shown) did not support phosphorylation. Since this suggests that in vivo the kinase may be regulated through a modulated PQ/PQH$_2$ ratio, we checked if activation of the kinase was directed by light absorbed in PS I (710 nm) and 650 nm light absorbed mainly by PS II [30]. Since 650 nm light is close to the red absorption maximum of chlorophylls, it resulted in identical phosphorylation levels of the 35 kDa protein as did white light, only at a lower intensity (Table I). Light absorbed by PS I yielded much less labelling of the 35 kDa protein, close to the level found for dark-

Table I

Percentage of radioactive label associated with the 35 kDa protein relative to the maximum found under red light conditions

Radioactivity was estimated from densitometry analysis of proteins in the 30 to 45 kDa range after correction for the background radiation level apparent on autoradiograms.

Treatment	Percentage of max. radioactive label
Dark	0
White light (150 quanta m^{-2} s^{-1})	96
Red light (650 nm) (20 quanta m^{-2} s^{-1})	100
Far red light (710 nm) (20 quanta m^{-2} s^{-1})	19.5

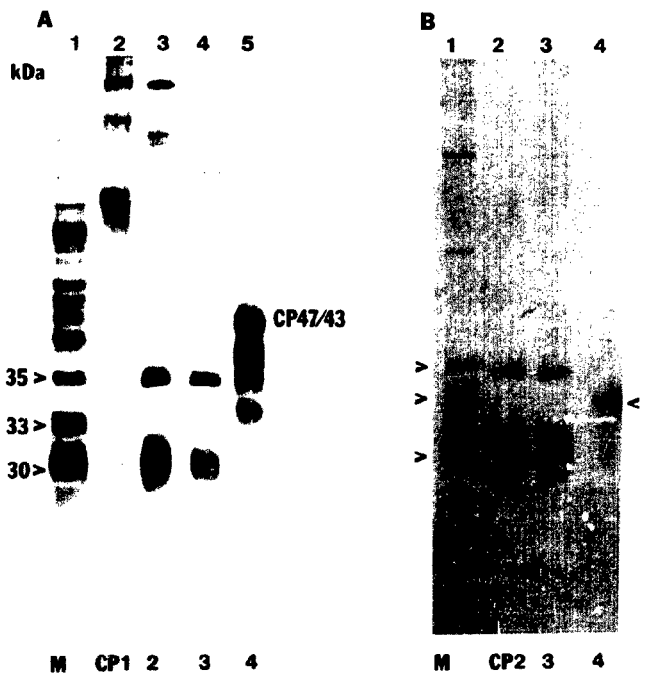

Fig. 8. Identification of chlorophyll *a*/*b* apoproteins following denaturing electrophoresis of chlorophyll protein complexes. Panel A: electrophoretic profile after Coomassie Blue staining of 1 thylakoid membranes; 2–5 polypeptide composition of CP1, 2, 3 and 4, respectively. CP4 contains PS II components as judged by their cross-reactivity to CP47 and CP43 antisera [28]. Panel B: immunoblot of membranes and CP2–4 probed with the antibody to the 30 kDa antenna protein [36]. 1 Immunodecoration of thylakoid membranes; 2–4 chlorophyll-protein complexes CP2–4, respectively.

treated cells. The intensity of far-red light was found to antagonize a state 2 situation in *P. hollandica* (Post, unpublished results). These data suggest that reversible phosphorylation of thylakoid proteins occurs in vivo and is most probably light/redox controlled.

Immunological relation of antenna proteins

Non-denaturing gel electrophoresis of Deriphat 160 extracts yielded four major green complexes, CP1–CP4, seen in the visible light (Fig. 7a). Upon transillumination with long-wavelength UV light, complex CP1 quenched fluorescence, a property diagnostic for the PS I reaction center. CP2–CP4 were highly fluorescent (Fig. 7b), indicating that these complexes were either antenna or PS II core components which lack an efficient quenching mechanism. Absorption spectroscopy identified CP2 and CP3 as the major antenna complexes, due to the presence of Chl *b* in these bands at a Chl *a*/*b* ratio of 2.5. Denaturing electrophoresis of CP2 and CP3 yielded two major proteins of 35 and 30 kDa (Fig. 8a). These two proteins were also present in the CP1 complex but were absent from CP4. A polyclonal antibody to the 30 kDa antenna polypeptide [36] recognized the 35 and 30 kDa polypeptides of bands CP2 and CP3, and also a 33 kDa polypeptide in CP4 (Fig. 8b). Previous studies have identified CP4 as containing proteins of the PS II core [28].

Identification of a reversibly phosphorylated PS I antenna apoprotein

The presence of redox-controlled kinase activity may indicate, in analogy to chloroplast systems, that an antenna component is the target for reversible phosphorylation. We identified on non-denaturing gels the Chl *a*/*b* antenna complexes after exposure to phosphorylating conditions in vivo. CP2 and CP3 were then separated by denaturing gel electrophoresis, and both complexes contained the 35 kDa antenna polypeptide which was identified as a phosphoprotein by autoradiography (Fig. 9a, b). We want to note here that the apparent absence of the 30 kDa antenna protein (Fig. 9a) is explained from a very poor reaction of this protein with silver stain, whereas the 35 kDa protein yielded a very strong reaction. The 30 kDa protein could be easily detected in Coomassie blue stain (Fig. 8a). In vitro phosphorylation of thylakoid membranes in the presence of NaF revealed that part of the radioactive label was not located in chlorophyll-protein complexes (Fig. 10a). Excised fraction 1 contained non-dissolved complexes. Fractions 2–4 (being green complexes CP 1–3) contained the 35 kDa phosphoprotein (Fig. 10c). Protein composition of the green complexes was similar to that shown in Fig. 8. Most of the radioactive label (Fig. 10b) was located in a complex containing the 23 and 25 kDa phosphoproteins (Fig 10c, bands 5, 6), and they were not part of a pigment-protein complex under these conditions. However, their phosphorylated state may help preserve a state 2 condition and therefore they may be localized in the direct environment of the photosynthetic antenna (Post, unpublished results).

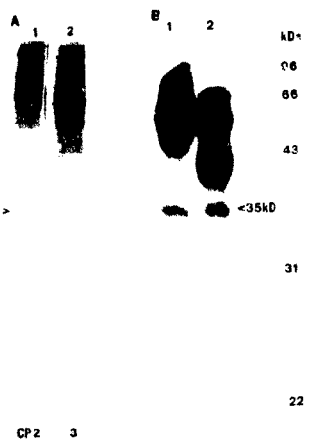

Fig. 9. The 35 kDa phosphoprotein is a chlorophyll *a*/*b* apoprotein. Panel A: silver-stained polypeptide composition of in vivo phosphorylated chlorophyll-protein complexes CP2 and 3 obtained from a non-denaturing gel as described in Fig. 7. Panel B: autoradiogram of lanes 1 and 2 of panel A. The radioactive label above the 35 kDa protein is due to the presence of nucleic acids, since they were not found after DNAase–RNAase treatment of Deriphat extracts.

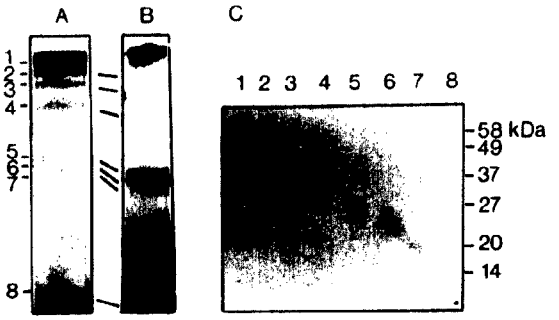

Fig. 10. The 23 and 25 kDa proteins are localized in a complex that does not bind chlorophylls. Panel A: non-denaturing gel of *P. hollandica* after in vitro phosphorylation in the presence of 10 mM NaF (left). Panel B: autoradiogram of the phosphorylated complexes. Panel C: autoradiogram of denaturing gel after SDS PAGE of bands excised and numbered as indicated in panel A. band 1, non-dissolved green complexes; band 2–4, CP1-CP3; band 5–7, non-pigmented complexes, band 8, free pigments.

Discussion

A membrane-bound kinase phosphorylating thylakoid proteins of 14, 23, 25 and 35 kDa, is present in *P. hollandica*. Thylakoids isolated according to stan-

dard procedures yielded a kinase constantly active in light and dark as reported previously for *Prochloron* [23], *P. hollandica* [29] and, e.g. *Acetabularia* [4]. The kinase activity in *P. hollandica* membranes in vitro, abolished by strong oxidizing agents [29], was irresponsive to the addition of quinone analogs (Fig. 2). Fluorescence kinetics (Fig. 3) and PS II dependent electron transport from H_2O to DCIP (data not shown) indicated the presence of intact PS II in isolated membranes with a PQ-pool almost completely oxidized in the dark. Partial inactivation of the kinase could be obtained by prolonged dark incubation prior to thylakoid isolation. In vivo the kinase could be activated by white light and by red light and it was inactivated in the dark and in far-red light. These observations lend evidence to the presence of a light/redox responsive protein kinase, which is slowly inactivated in the dark. The major target for phosphorylation was a 35 kDa protein which was identified as an apoprotein of the Chl *a/b* antenna. In an earlier paper [36], we demonstrated that the 30 and 35 kDa proteins form a Chl *a/b* antenna and are immunologically related to one another and to the 34 kDa antenna protein of *Prochloron*. The 34 kDa Chl *a/b* binding protein of *Prochloron* is phosphorylated in vitro in a light-independent manner and hence reversible phosphorylation of antenna apoproteins was rejected as the basis for state transitions in vivo [23]. The data presented here indicate that the *Prochloron* sp. kinase may behave similar to that of *P. hollandica* and could be in fact light-regulated.

Antimycin A enhanced the in vitro phosphorylation of 23 and 25 kDa thylakoid proteins in the light. A similar effect was observed on phosphorylation of LHC II in *Acetabularia* [4]. According to [37] this suggests, that cyclic electron flow may be involved in controlling levels of phosphorylation in *Prochlorothrix*. The 23 and 25 kDa phosphoproteins are as yet unidentified but shown not to be part of green complexes. The 14 kDa phosphoprotein found in *P. hollandica* thylakoids may be similar to the 15 kDa cyanobacterial thylakoid protein which undergoes reversible phosphorylation [19].

Solubilization of prochlorophyte thylakoids yields a large part of the major Chl *a/b* antenna complex, containing the 35 kDa phosphoprotein, in a free form. The 35 kDa protein was not detected in complexes containing PS II. Part of the antenna copurified with PS I irrespective whether solubilization was done with SDS [26] or octyl β-D-glucopyranoside/sodium cholate [38] in the case of *Prochloron*, or with Triton X-100 [39], dodecyl maltoside [28], LDS or Deriphat 160 (this paper) in the case of *P. hollandica*. Zwittergent 14 is so far the only reported detergent which successfully solubilizes the Chl *a/b* antenna separately from PS I [39]. In parallel, Chl *b* was found to copurify with PS I [26] and direct energy coupling between Chl *b* and PS I was shown in fractionated thylakoids [28]. The finding that the abundant 35 kDa Chl *a/b* antenna apoprotein undergoes reversible phosphorylation upon red/far-red illumination is especially interesting in this respect. State 1–2 transitions have been shown to take place upon transfer of cells to red light and this effect was antagonized by far-red light [30]. We hypothesize that the 35 kDa apoprotein is part of an antenna complex structurally associated with PS I. Reversible phosphorylation of the 35 kDa apoprotein may be functional in changing the orientation of the Chl *a/b* antenna towards PS I, thereby modulating direct energy transfer from this antenna complex to PS II. The involvement of PS I antenna complexes in the regulation of excitation energy distribution and balanced photosynthesis in *P. hollandica* was part of a recent study.

Our α-30 kDa antibody identified a new polypeptide of 33 kDa which migrated in a PS II-associated complex. This raises the possibility that in vivo the 30 and 35 kDa proteins form a PS I antenna, whereas the 33 kDa polypeptide is coupled to PS II. Of the three Chl *a/b* proteins the kinase phosphorylates only one, the 35 kDa apoprotein which copurifies with PS I. The 35 kDa polypeptide is not a phosphorylated form of the 30 and 33 kDa protein as determined from comparison with protein patterns of non phosphorylated dark-adapted cells.

Acknowledgements

This work was supported by grant No. 88-00341 to I.O. and A.F.P. obtained from the Israel-USA binational research foundation and National Science Foundation grant DCB-8903060 to G.S.B. A.F.P. was supported by a special research grant from the Royal Dutch Academy of Sciences, Netherlands.

References

1 Telfer, A., Allen, J.F., Barber, J. and Bennett, J. (1983) Biochim. Biophys. Acta 722, 176–181.
2 Malkin, S., Telfer, A. and Barber, J. (1980) Biochim. Biophys. Acta 848, 48–57.
3 Satoh, K. and Fork, D.C. (1983) Photochem. Photobiol. 37, 429–434.
4 Gal, A., Schuster, G., Frid, D., Canaani, O., Schwieger, G. and Ohad, I. (1988) J. Biol. Chem. 263, 7785–7791.
5 Ley, A.C. and Butler, W. (1976) Proc. Natl. Acad. Sci. USA 73, 3957–3960.
6 Satoh, K. and Fork, D.C. (1983) Biochim. Biophys. Acta 722, 190–196.
7 Biggins, J., Campbell, C.L. and Bruce, D. (1984) Biochim. Biophys. Acta 767, 138–144.
8 Snyder, U.K. and Biggins, J. (1987) Biochim. Biophys. Acta 892, 48–55.
9 Fork, D.C., and Satoh, K. (1983) Photochem. Photobiol. 37, 421–427.
10 Dominy, P.J. and Williams, W.P. (1987) Biochim. Biophys. Acta 892, 264–274.

11 Canaani, O. (1986) Biochim. Biophys. Acta 852, 74–80.
12 Post, A.F., Veen, A. and Mur, L.R. (1986) FEMS Microbiol. Lett. 35, 129–133.
13 Kyle, D.J., Kuang, T.Y., Watson, J.L. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 89–96.
14 Horton, P. and Black, M.T. (1980) FEBS Lett. 119, 141–144.
15 Gal, A., Schachak, Y., Schuster, G. and Ohad, I. (1987) FEBS Lett. 223, 205–210.
16 Wollman, F.A., and Lamarre, C. (1988) Biochim. Biophys. Acta 933, 85–94.
17 Coughlan, S.J. (1988) Biochim. Biophys. Acta 933, 413–422.
18 Allen, J.F., Sanders, C.E. and Holmes, N.G. (1985) FEBS Lett. 193, 271–275.
19 Harrison M.A., Tsinorema N.F. and Allen J.F. (1991) FEBS Lett. 282; 295–299.
20 Bruce, D., Biggins, J. Steiner, T. and Thewalt, M. (1985) Biochim. Biophys. Acta 806, 237–246.
21 Mullineaux, C.W. and Allen, J.F. (1990) Photosynthesis Res. 23, 297–311.
22 Lewin, R.A. (1986) Gi. Bot. Ital. 120, 1–14.
23 Schuster, G., Owens, G.C., Cohen, Y. and Ohad, I. (1984) Biochim. Biophys. Acta 767, 596–605.
24 Withers, N., Vidaver, W. and Lewin, R.A. (1978) Phycology 17, 167–171.
25 Withers, N.W., Alberts, R.S., Lewin, R.A., Thornber, J.P., Britton, G. and Goodwin, T.W. (1978) Proc. Natl. Acad. Sci. USA 75, 2301–2305.
26 Hiller, R.G. and Larkum, A.W.D. (1985) Biochim. Biophys. Acta 806, 107–115.
27 Burger-Wiersma, T., Veenhuis, M., Korthals, H.J., Van de Wiel C.C.M. and Mur, L.R. (1986) Nature 320, 262–264.
28 Bullerjahn, G.S., Matthijs, H.C.P., Mur, L.R. and Sherman, L.A. (1987) Eur. J. Biochem. 168, 295–300.
29 Matthijs, H.C.P., Van der Staay, G. and Mur, L.R. (1989) Biochim. Biophys. Acta 975, 317–324.
30 Burger-Wiersma, T. and Post, A.F. (1989) Plant Physiol. 91, 770–774.
31 Rippka R., Dereuilles J., Waterbury, J.B., Herdman M. and Stanier R.U. (1979) J. Gen. Microbiol. 111, 1–61.
32 Towbin, H., Stachelin, T. and Gordon J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
33 Smith, D.E. and Fisher, P.A. (1984) J. Cell Biol. 99, 20–26.
34 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
35 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
36 Bullerjahn, G.S., Jensen, T.C., Sherman, D.M. and Sherman, L.A. (1990) FEMS Microbiol. Lett. 67, 99–106.
37 Moss, D.A. and Bendall, D.S. (1984) Biochim. Biophys. Acta 767, 389–395.
38 Schuster, G. Nechustai, R., Nelson, N. and Ohad, I. (1985) FEBS Lett. 191, 29–33.
39 Matthijs, H.C.P. Reith, H. and Van Der Staay, G.W.M. (1989) in Current Research in Photosynthesis, Vol. II (Baltscheffsky M., ed.), pp. 201–204. Kluwer.