Biochimica et Biophysica Acta, 767 (1984) 596–605
Elsevier

BBA 41662

THYLAKOID POLYPEPTIDE COMPOSITION AND LIGHT-INDEPENDENT PHOSPHORYLATION OF THE CHLOROPHYLL *a,b*-PROTEIN IN *PROCHLORON*, A PROKARYOTE EXHIBITING OXYGENIC PHOTOSYNTHESIS

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(Received June 29th, 1984)

Key words: Protein kinase; Protein phosphorylation; Chlorophyll fluorescence; Chlorophyll-protein complex; Photosynthesis; (Prochloron, Fremyella)

Thylakoids of the prokaryote *Prochloron*, present as a symbiont in ascidians isolated from the Red Sea at Eilat (Israel), showed polypeptide electrophoretic patterns comparable to those of thylakoids from eukaryotic oxygen-evolving organisms. Low temperature, fluorescence spectroscopy of *Prochloron*, having a chlorophyll *a/b* ratio of 3.8–5, and frozen in situ, demonstrated the presence of Photosystem II chlorophyll-protein complex emitting at 686 and 696 nm, as well as the emission band of Photosystem I at 720 nm which was so far not observed in *Prochloron* species. The latter emission was absent, if the cells or thylakoids were isolated prior to freezing. Energy transfer from chlorophyll *b* to chlorophyll *a* could be demonstrated to occur in vivo. The chlorophyll *a,b*-protein complex of Photosystem II, isolated by non-denaturing polyacrylamide gel electrophoresis, contained one major polypeptide of 34 kDa. The polypeptide was phosphorylated in vitro by a membrane-bound protein kinase which was not stimulated by light. A light-independent protein kinase activity was also found in isolated thylakoids of another prokaryote, the cyanophyte *Fremyella diplosiphon*. State I–State II transition could not be demonstrated in *Prochloron* by measurements of modulated fluorescence intensity in situ. We suggest that the presence of a light-independent thylakoid protein kinase of *Prochloron*, collected in the Red Sea at not less than 30 m depth, might be the result of an evolutionary process whereby this organism has adapted to an environment in which light, absorbed preferentially by Photosystem II, prevails.

Introduction

The discovery of the oxygen-evolving, chlorophyll *b*-containing prokaryote, *Prochloron* [1], has aroused wide interest and has promoted much speculation with regard to its place in the evolution of the photosynthetic apparatus [2–8]. Our interest in this organism was prompted by the finding that its chlorophyll *b* is organized in a chlorophyll *a,b*-protein complex [6] and that the structural organization of its thylakoids is very similar to that of green algae [7]. It is now well established that modulation of energy distribution between the two photosystems in green algae and higher plants is regulated by a process of phosphorylation-dephosphorylation of the light-harvesting chlorophyll *a,b*-protein complex [9]. The usefulness of such a regulatory system is evident, if

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light-harvesting chlorophyll *a,b*-protein complex; CPI, chlorophyll *a*-protein complex I; CPII, chlorophyll *a,b*-protein complex II, Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

one considers the continuous variation in both the intensity and quality of the light available to the leaves of various parts of a plant in a densely plant populated area [9]. On the other hand, *Prochloron* cells are found as symbionts in ascidians living up to 40 m depth in various oceans from Australia to the Red Sea [6,10], where the light intensity is rather constant and blue light (400–650 nm) predominates. Thus, it was of interest to examine whether modulation of energy distribution between the two photosystems, as expressed by State I → State II transition [11,12] occurs in *Prochloron* and whether such modulation is achieved by a light-dependent protein kinase activity, as reported for higher plants [9,13–17].

In the present work, the photosynthetic activity, thylakoid polypeptide composition and phosphorylation in a *Prochloron* species, isolated from the Red Sea at Eilat, have been studied. For comparison, the thylakoid phosphorylation in vivo and in vitro of a cyanobacterium prokaryote, *Fremyella diplosiphon*, in which phycobilisomes serve as the major light-harvesting antenna of Photosystem II, have also been determined. Part of the results presented here were the subject of a preliminary presentation [10].

Materials and Methods

Cells

Prochloron cells were isolated from the didemnid *Diplosoma virens* collected at the Gulf of Aqaba, near the Marine Biological Laboratory in Eilat, Israel, at a depth of about 30 m by scuba diving. The in situ light intensity was 20–40 $J \cdot m^{-2} \cdot s^{-1}$. The organism was kept in running sea water until isolation of the algal cells (3–8 h). Cells were released from the ascidians by gentle pressure and flushing with sea water containing 10 mM Tricine buffer (pH 8.0) using a Pasteur pipette. The cells were collected from the flushing liquid by centrifugation at $3000 \times g$ for 2 min at 4°C. The pelleted cells were resuspended and washed in a buffer containing 50 mM Tris-HCl (pH 8.0), 100 mM sorbitol, 10 mM $MgCl_2$ and 2 mM benzamidine. For preparation of thylakoids, the cells were disrupted in the same buffer by passing through a French pressure cell operated at 3500 lb/in^2 at 0°C. The homogenate (5 ml) was layered on a sucrose solution (60% in the same buffer) in a 10-ml polycarbonate tube of the Beckman 40 rotor, and centrifuged at $100\,000 \times g$ for 5 min. The membrane fraction at the buffer-sucrose interphase was collected, washed by centrifugation in buffer as above, and stored at −80°C until further use.

F. diplosiphon cells were grown as described by Kirilovsky et al. [18]. For in vivo labeling experiments, cells were grown for 24 h in the presence of [^{32}P]orthophosphate. The specific activity of [^{32}P]orthophosphate utilized was 17 Ci/mol. Labeled cells were disrupted by sonication at 4°C (Branson Sonifier, setting 5, 3 × 15 s, with intervals of 15 s) in phosphate-citrate buffer (0.5, 0.3 M, respectively, pH 7.0). Purified membrane-bound phycobilisomes were prepared as described earlier [18]. Phycobiliprotein-free thylakoids were obtained from such preparations, following resuspension in Tris-HCl buffer (50 mM, pH 8.0) for 15 min and centrifugation of the resulting dissociated mixture of phycobiliproteins and membranes at $100\,000 \times g$ for 15 min at 4°C. The pelleted membranes were washed, as above, and kept frozen at −80°C until use. *Chlamydomonas reinhardtii* cells and thylakoids were prepared as described by Owens and Ohad [19].

Methods

Phosphorylation of membrane polypeptides in vitro was carried out as described previously [19]. Isolated thylakoids were incubated in the light or dark, for 10 min at 25°C, in a reaction mixture containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$ and 0.15 Ci/mmol [γ-^{32}P]ATP.

Measurements of variable fluorescence were carried out using a modulated light source, as described by Bults et al. [20]. State I → State II transitions were recorded as described before [21].

Fluorescence emission and excitation spectra at 77 K were performed as described by Kirilovsky et al. [18]. Low-temperature absorption spectra (77 K) were obtained by using the attachment of the dual-wavelength spectrophotometer of Aminco-Chance. Measurements of oxygen evolution in intact cells were carried out by use of microelectrodes inserted directly into the *Prochloron*-containing region of ascidian colonies in vivo [22,23].

Separation of chlorophyll-protein complexes

was performed by polyacrylamide gel electrophoresis, using LDS, at 4°C, according to Delepelaire and Chua [24] or by the method described by Markwell et al. [25]. Resolution of thylakoid polypeptides by denaturing gel electrophoresis in the presence of LDS at 4°C was carried out as described by Laemmli [26].

Measurements of chlorophyll concentrations were done as described by Arnon [27].

Results

Spectral properties of intact Prochloron cells and chlorophyll-protein complexes

The absorption spectrum of intact cells shows the presence of major peaks at 443 and 673 nm. Low-temperature spectra (77 K) disclose also the presence of chlorophyll *b* (654 nm) (Fig. 1). The ratio chlorophyll *a*/*b* in various preparations was 3.8–5.0.

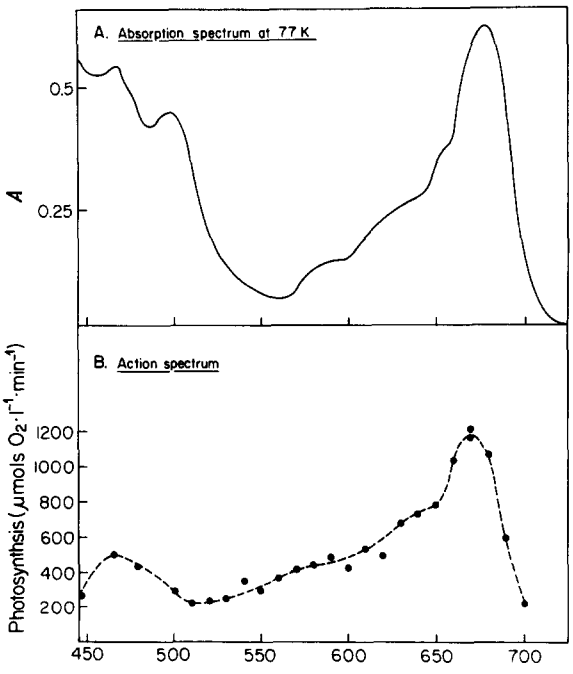


Fig. 1. (A) Absorption spectrum at 77 K of *Prochloron* cells. (B) Action spectra in vivo of an intact colony of *Diplosoma virens* containing *Prochloron*. Oxygen-evolution rates were determined according to Jorgensen et al. [23], using a combined oxygen microelectrode of a sensing tip of 12 μm introduced into the colony by a micromanipulator under a dissection scope. The microelectrode was manufactured according to Revsbech and Ward [36]. Light was provided by a Schott KL 1500 point illuminator, guided by a 6 mm optic fiber and filtered through a 40 cm long Zeiss continuous interference filter (405–710 nm). Light saturation curves were carried out at wavelengths of 440, 578, 628, 668 and 704 nm. Action spectra were determined under light limitations observed at the above indicated wavelengths.

The fluorescence emission spectrum at 77 K is characterized by two emission maxima at 686 and 696 nm, excited by chlorophyll *a* absorbing at 670 nm and chlorophyll *b* absorbing at 650 nm (Fig. 2). The low-temperature fluorescence emission spectrum of isolated cells or thylakoids did not show the 715–740 nm peak ascribed to the chlorophyll-protein complex of Photosystem I (Fig. 2). Similar results have been reported before [28]. However, when intact ascidian colonies, still attached to the coral support, were quickly frozen in liquid nitrogen, the 77 K fluorescence emission showed a large 720 nm emission band (Fig. 2), as expected for a photosynthetically active oxygen-evolving organism. Furthermore, this fluorescence emission band was absent in colonies of the ascidians devoid of *Prochloron* cells.

Separation of chlorophyll-protein complexes, using the method described by Delepelaire and Chua [24], showed one major green band of the chlorophyll *a*-protein complex CPI (Fig. 3). The

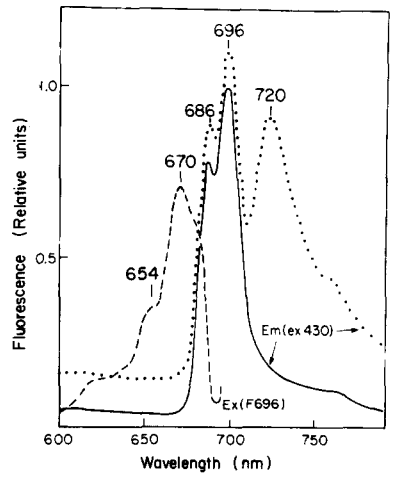


Fig. 2. Low-temperature (77 K) fluorescence emission and excitation spectra of *Prochloron* cells. ————, Emission (Em) of intact isolated cells (excitation was at 430 nm); — — —, excitation (Ex) spectrum of the 696 nm emission peak (a similar spectrum was obtained for the 686 nm emission peak); ·····, emission spectrum of *Prochloron* cells in situ before removal from the ascidian host (excitation was at 430 nm).

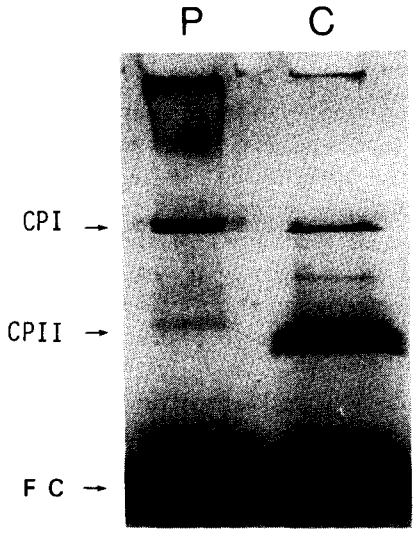


Fig. 3. Chlorophyll-protein complexes of *Prochloron* (P) and *Chlamydomonas reinhardtii* (C) separated according to Delepelaire and Chua [24]. The chlorophyll-protein complex CPII of *Prochloron* was not stable and the chlorophyll dissociated from the binding polypeptides during the electrophoretic separation. To minimize this effect, the run was stopped after about 40 min when the CPII of *Prochloron* could still be detected. CPI, chlorophyll-protein complex I; CPII, chlorophyll-protein complex II; FC, dissociated free chlorophyll.

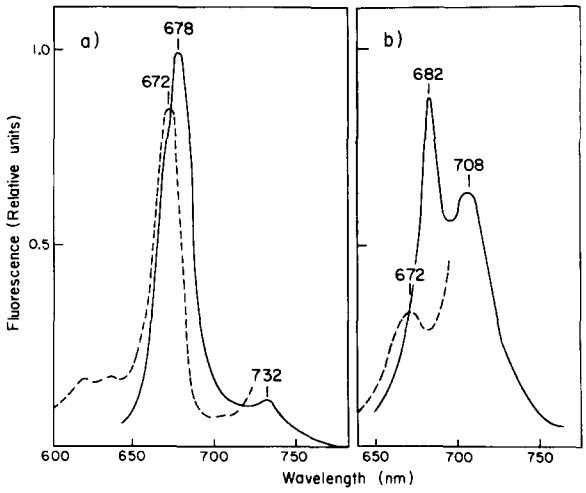


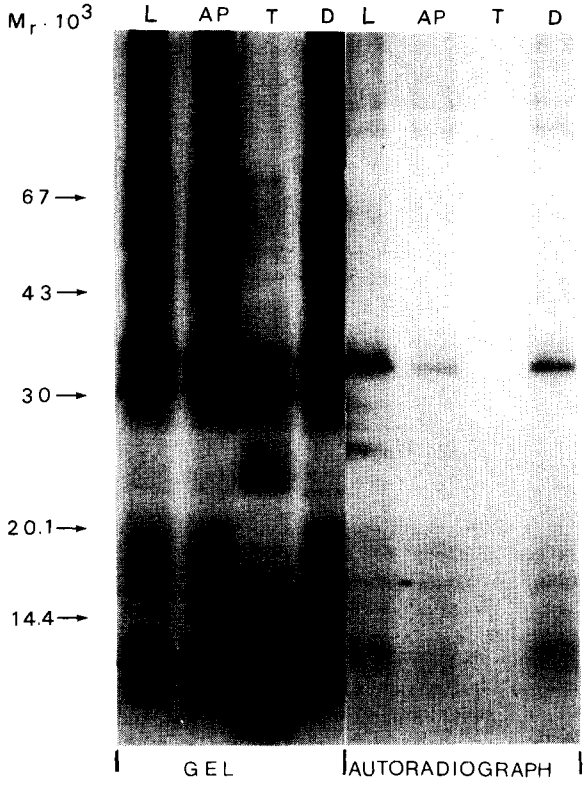
Fig. 4. Low-temperature fluorescence (77 K) spectrum of *Prochloron* chlorophyll-protein complexes: CPII (a) and CPI (b). Chlorophyll-protein complexes were separated as in Fig. 3. Gel slices, 1 mm width, sectioned from the regions containing the various complexes, were frozen in liquid nitrogen and the spectra were recorded as described in Materials and Methods. ————, Emission spectrum, excitation was at 430 nm; - - - - - -, excitation spectrum of the emission bands of 732 and 708 nm of CPII and CPI, respectively. The peak of excitation spectrum of the 678 nm band in (a) was similar to that of the 732 nm emission.

CPII complex was unstable and dissociated when electrophoresis was carried out for longer than 30–40 min, even at 4°C. For comparison, the chlorophyll-protein complexes of *Chlamydomonas* were resolved on the same gel, and show the typical Photosystem II chlorophyll *a* complexes CPIII-IV and CPII resolved here, after a short run, only into three bands. Following longer runs, the CPII of *Chlamydomonas* can be resolved into five bands [24].

The fluorescence emission and excitation spectra of the CPII and CPI complexes are shown in Fig. 4. The CPII complex isolated by this method is partially denatured and shows a 77 K fluorescence emission at 678 nm, as compared to the usual 680 nm emission. The peak excitation of this emission is at 672 nm (chlorophyll *a*), but no detectable excitation by chlorophyll *b* (653 nm) was observed, indicating that chlorophyll *b* initially present in this complex is dissociated from and does not transfer energy to chlorophyll *a*. The 77 K fluorescence emission spectrum of CPI exhibits peaks at 682 and 708 nm, both excited by chlorophyll *a*, with a peak absorption at 672 nm (Fig. 4). Analysis of the chlorophyll-protein complexes of *Prochloron* by the electrophoretic system of Markwell et al. [25] could resolve five different chlorophyll-protein complexes, two of them containing only chlorophyll *a* and three chlorophyll *a* and *b*, as reported for higher plants [25] (data not shown).

Polypeptide composition and in vitro phosphorylation of Prochloron thylakoids

The electrophoretic pattern of *Prochloron* thylakoids is characterized by the absence of distinct polypeptide bands in the molecular mass range of 22–28 kDa (Fig. 5). The major polypeptides of *Prochloron* thylakoids are found at 34, 30, 44, 47 and 68 kDa. Minor bands can also be detected at 9–18 kDa and 55–60 kDa (Fig. 5). To determine whether polypeptides of *Prochloron* thylakoids are phosphorylated by a membrane-bound kinase, isolated thylakoids were incubated



Fiig. 5. In vitro phosphorylation of isolated *Prochloron* thylakoids. L, incubation in light for 10 min; AP, incubation with alkaline phosphatase (Sigma, *Escherichia coli*, 30 units/ml, 15 min at 25°C) after phosphorylation in the light; T, incubation with trypsin (Sigma, bovine pancreas, 50 μg/ml, 15 min at 25°C) after phosphorylation in the light; D, incubation in the dark for 10 min; phosphorylation was carried out as described in Materials and Methods.

with [γ-^{32}P]ATP. Only one major phosphorylated polypeptide band, coinciding with the 34 kDa polypeptide, was obtained following electrophoresis of in vitro ^{32}P-labeled thylakoids. Polypeptide phosphorylation in vitro of *Prochloron* thylakoids by the membrane-bound kinase was not significantly stimulated by light; basically a similar phosphorylation pattern was obtained for membranes incubated in the light or dark (Fig. 5).

Treatment of in vitro phosphorylated thylakoids with alkaline phosphatase removed most of the 34 kDa polypeptide phosphate. Treatment of thylakoids with trypsin removed most but not all the stainable 34 kDa polypeptide and all the stainable polypeptide bands of an apparent molecular mass higher than 34 kDa (Fig. 5). Distinct stainable digestion products of thylakoids could be detected by gel electrophoresis. Smaller degradation products (not more than 10 kDa) appeared as a diffusely spread band. Trypsinization caused complete disappearance of the ^{32}P radioactivity from all the remaining polypeptides on the gel. The phosphorylated segment(s) of the 34 kDa phosphopeptide generated by trypsin cleavage could not be detected in the electrophoretic system used, which could resolve polypeptides of not less than 5 kDa (Fig. 5).

Analysis of the polypeptide pattern of *Prochloron* chlorophyll-protein complexes, separated as described before [24], showed that the phosphorylated 34 kDa polypeptide was present only in the CPII complex. As mentioned above, the CPII complex was unstable and the chlorophyll dissociated from the chlorophyll-binding polypeptides during electrophoretic separation. In order to reduce the degree of CPII dissociation, electrophoresis of in vitro phosphorylated thylakoids was carried out for short periods of time. Following a short electrophoretic run (not more than 20 min), the CPII complex was resolved into two bands, referred to as upper CPII ($CPII_U$) and lower CPII ($CPII_L$). Analysis of the polypeptide composition of these CPII green bands by re-electrophoresis under denaturing conditions, showed that the upper band contained the phosphorylated 34 kDa polypeptide, while the lower band contained, in addition to traces of the 34 kDa polypeptide, distinct phosphopeptides of about 32, 30, 28 and 26 kDa. Comparison of the amount of ^{32}P radioactivity of these peptides with their Coomassie brilliant blue staining showed that the lower peptides were more radioactively labeled than the upper ones (Fig. 6).

Photosynthetic activity of Prochloron cells in situ

Measurements of photosynthetic oxygen evolution were carried out by inserting an oxygen microelectrode into an intact *Prochloron*-containing ascidian colony immersed in continuously aerated sea water. The action spectrum of this activity is depicted in Fig. 1. Oxygen evolution elicited by 450–550 nm light was low, as compared to the absorption intensity of the intact cells, but coincided well with the absorption spectrum at wave-

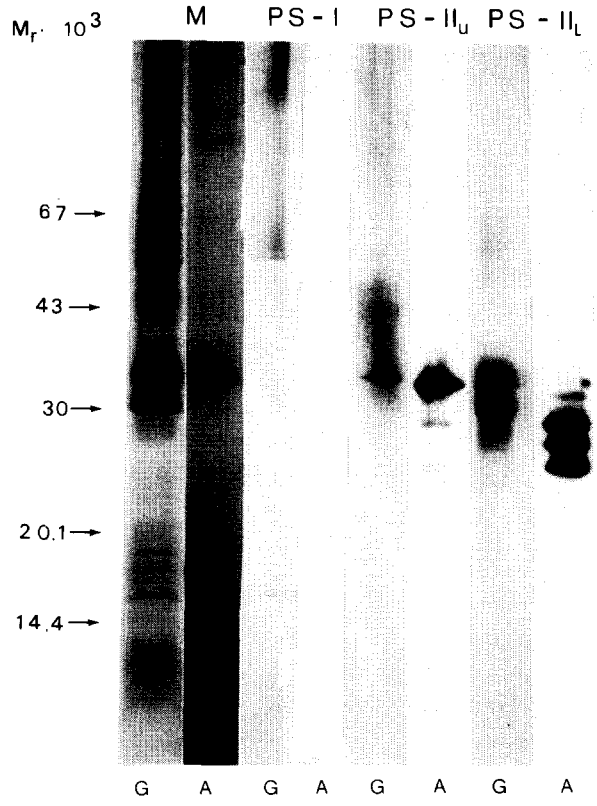


Fig. 6. Presence of phosphorylated polypeptides in chlorophyll-protein complexes of *Prochloron*. Isolated membranes were phosphorylated in vitro, and chlorophyll-protein complexes were resolved as in Fig. 3. Gel slices corresponding to the chlorophyll-protein complexes were re-electrophoresed under denaturing conditions by LDS-polyacrylamide gel electrophoresis. G, gel; A, autoradiograph; M, membranes. When a non-denaturing gel was run for a short time, the chlorophyll-protein complex II was resolved into two bands, namely, upper ($CPII_U$) and lower ($CPII_L$).

lengths from 600 to 700 nm. Energy transfer from chlorophyll *b* to the photosynthetic reaction centres was evident when comparing the absorption and action spectra for photosynthesis (Fig. 1A, B; see also Fig. 2).

Measurements of State I → State II transition were carried out with intact *Prochloron* cells in situ as above. Modulated blue light (480 nm; 4.4 $J \cdot m^{-2} \cdot s^{-1}$) was used for excitation of chlorophyll *b*, and the emitted modulated fluorescence was recorded by using a lock-in amplifier as described before [16,20,21]. Addition of continuous saturating amount of 710 nm light did not elicit appreciable changes in the modulated fluorescence intensity emitted at 686 nm. No reduction of the fluorescence intensity was observed following continuous illumination for 15–20 min (Fig. 7). The same results were obtained when the added continuous light was of 700 or 690 nm. To ascertain that state transition could be detected with this experimental set-up, *Chlamydomonas* cells were used in a similar experiment, and a reduction of approx. 20–30% in the intensity of the modulated fluorescence was observed after 10 min, as expected [16,17,21]. Addition of 710 nm continuous saturating light induced an immediate reduction of the modulated fluorescence; this is expected, if Photosystem I becomes saturated and Photosystem II operates under limiting light conditions. The *Prochloron* cells exposed to blue modulated light in situ remained photosynthetically active at the end of the above-described experiments, as shown by the change in fluorescence kinetics upon addition of DCMU (Fig. 7, inset).

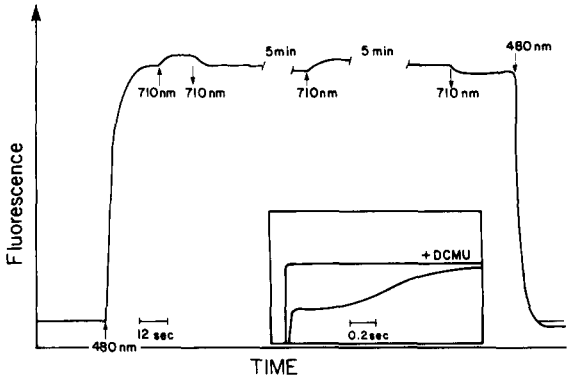


Fig. 7. Relative changes in the yield of modulated chlorophyll fluorescence from *Prochloron* in situ. Intact ascidian colonies, attached to a fragment of coral support, were dark-adapted for 30 min. Illumination with modulated (23 Hz) light II (480 nm; 4.4 $J \cdot m^{-2} \cdot s^{-1}$) caused appearance of modulated chlorophyll fluorescence measured at 680 nm. Addition of light I (710 nm; 8 $J \cdot m^{-2} \cdot s^{-1}$) did not elicit appreciable changes in the modulated fluorescence intensity. No reduction in the fluorescence intensity due to State I–State II transitions was observed. No change was observed when light I was 690 or 700 nm and the intensity was varied 6–10 $J \cdot m^{-2} \cdot s^{-1}$. Starting from a state of preillumination with light I or light I + light II gave the same results. Inset: Fluorescence induction curve of intact cells of *Prochloron* in situ.

Phosphorylation of thylakoid polypeptides in the cyanobacterium F. diplosiphon

When *F. diplosiphon* cells were grown in the presence of [^{32}P]orthophosphate, three thylakoid proteins: one of 14.5 kDa and a doublet of 21 kDa, were phosphorylated (Fig. 8). Autoradiography of phosphorylated thylakoid preparations, hydrolyzed in HCl (6 M) in vacuo for 3 h and chromatographed on Whatman 3 MM paper, revealed the presence of phosphothreonine and phosphoserine. The existence of protein kinase activity in the cyanobacterial photosynthetic membrane was tested when mixtures of isolated purified membranes, with or without addition of dissociated phycobilisomes, were incubated with [γ-^{32}P]ATP. In contrast to the phosphorylation pattern in vivo, label was incorporated into a large number of polypeptides (Fig. 8). The membrane-associated protein kinase phosphorylated also exogenous substrates such as histone 2A (Fig. 8), and was active in light or dark. The difference in the phosphorylation pattern between intact cells and isolated thylakoids might reside in the fact that in vivo the phycobilisomes are attached to the membrane and might restrict the interaction of the kinase with various polypeptides which otherwise are exposed to its activity. Phosphorylation in vitro could not be assessed with intact phycobilisome-membrane complexes, since these are stable only in the presence of 0.5 M phosphate and 0.3

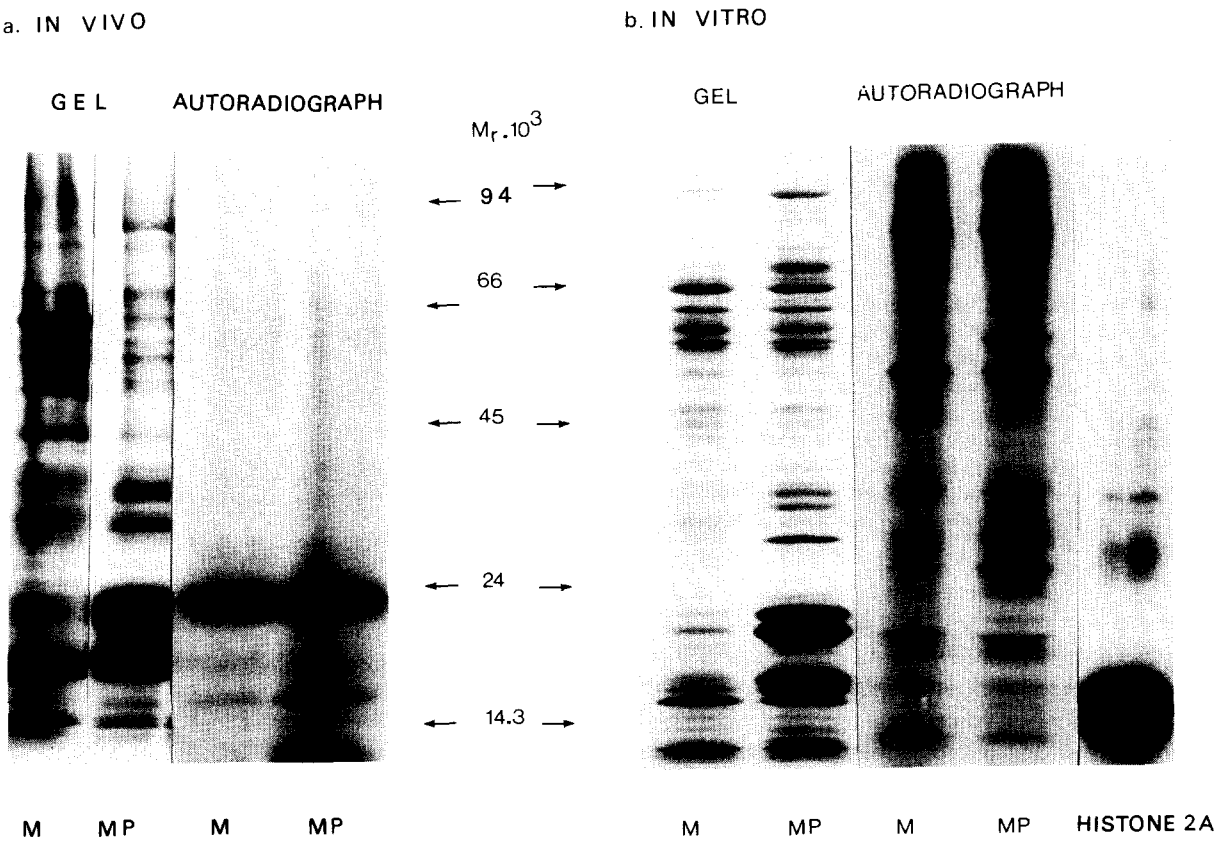


Fig. 8. Phosphorylation of *F. diplosiphon* thylakoids. (a) Incorporation of ^{32}P into thylakoid polypeptides of cells grown in white light in the presence of [^{32}P]orthophosphate for 24 h. Membranes without (M) or with bound phycobilisomes (MP) were prepared and the polypeptides resolved as described in Materials and Methods. (b) Phosphorylation of isolated thylakoids in vitro. Thylakoids without (M) or with addition of dissociated phycobilisomes (MP) were incubated with [γ-^{32}P]ATP in the light. The polypeptides were resolved by electrophoresis as above. Similar results were obtained when incubation was carried out in the dark. Histone 2A was added as an exogenous substrate to the membranes.

M citrate [18], which completely block the activity of the kinase.

Discussion

The results presented in this work show that the *Prochloron* species, collected from the Red Sea at Eilat, is quite similar to other *Prochloron* species so far reported from various Pacific Ocean isolates [6,28,29] in terms of their absorption spectra, presence of chlorophyll *b* and photosynthetic activity. Larkum and Hiller [29] reported a consistent difference in the chlorophyll *b* content between cells isolated in the summer and winter. This might explain the variation in the chlorophyll *a*/*b* ratio observed in this work which was carried out with cells collected over a period of about one and a half years.

The 77 K fluorescence emission spectra of isolated cells so far reported (Hiller, R.C. and Larkum, A.W.D., personal communication), as well as in this work, showed only the bands characteristic of Photosystem II (685 and 696 nm) [28]. This observation is apparently at variance with the findings and model proposed by Withers et al. [6], who reported the presence of a chlorophyll-protein complex I (CPI) usually characterized by a 77 K fluorescence emission band at 715–740 nm. The results presented in this work demonstrate the presence of this emission band at 720 nm in vivo which is, however, quenched following isolation of the cells or thylakoids.

The 77 K fluorescence emission of the isolated CPI complex of *Prochloron* as reported here, shows a peak at 708 nm. This emission could arise from the light-harvesting I complex when disconnected from the reaction centre I, as was reported from *Chlamydomonas* [30]. If this were the case, the in vivo organization of the Photosystem I chlorophyll in *Prochloron* might be similar to that of green algae, and consist of a reaction centre-core antenna complex emitting at 77 K at 720 nm and a light-harvesting I antenna emitting at 708 nm.

Analysis of the polypeptide pattern of *Prochloron* thylakoids demonstrated the presence of two major polypeptide bands at 34 and 30 kDa. The first band was present in all preparations, while the second band varied in its intensity in various preparations. Only the upper band (34 kDa) was found to be phosphorylated when isolated thylakoids were incubated in vitro with [γ-^{32}]ATP, and it was the major polypeptide of CPII. We therefore suggest that the 34 kDa polypeptide is the apoprotein of the phosphorylated chlorophyll *a*,*b*-protein light-harvesting complex (CPII) and that the 30 kDa band might be a degradation product of the 34 kDa polypeptide from which the phosphorylated segment was removed by cellular proteinases during isolation of the thylakoids. The apparent molecular weight of this polypeptide is higher than that of the polypeptides participating in the formation of the CPII complex in green algae or higher plants which have an apparent M_r between 24000 and 30000 [31].

In addition to the major phosphopeptide of 34 kDa, 3–4 minor phosphorylated bands of 24–30 kDa were observed, which could be degradation products of the major 34 kDa polypeptide. This conclusion is supported by the observation that following electrophoresis under denaturing conditions of the isolated chlorophyll *a*,*b*-protein complex (CPII) obtained from phosphorylated thylakoids, larger amounts of these fragments are generated, possibly by a detergent-activated proteinase(s) which cleaves the 34 kDa phosphopeptide at specific sites away from the surface-exposed polypeptide segment containing the esterified phosphate.

The most striking observation of the present work is the fact that the membrane-bound protein kinase which phosphorylates the major protein of CPII of *Prochloron* is not light-activated, as is the case for the enzyme in isolated thylakoids of green algae [19] and higher plants [9,13,14]. The possibility that the kinase remained active in the dark, following previous illumination, is ruled out by the fact that in some experiments the thylakoids were used after incubation at 4°C for 1 h in complete darkness. The dark de-activation of such kinases in higher plants and algae was reported to occur within 10–20 min [32].

It is interesting to consider that the light-dependent modulation of intersystem energy distribution might have appeared at later stages of the chloroplast evolution. The presence of a membrane-bound protein kinase which is not stimulated by light, also in the cyanophyte *Fremyella* as found in this work, might support this view. Until

now, thylakoid phosphorylation has not been demonstrated in cyanophytes. Phosphorylation of *Fremyella* thylakoids could be demonstrated only, if the cells were actually grown in the presence of $^{32}PO_4^{3-}$ for at least one generation (Owens, G.C. and Ohad, I., unpublished data), as opposed to only 20–40 min of incubation required for polypeptide phosphorylation in non-dividing algae such as *Chlamydomonas* [19]. The thylakoid-bound kinase(s) of *Fremyella* does not show the light activation and substrate specificity characteristic of the higher plants and green algae kinase involved in the regulation of energy distribution. Furthermore, cyanophytes do not possess a membrane-intrinsic light-harvesting chlorophyll *a*,*b*-protein complex. The major light-harvesting antennae of these organisms are the phycobilisomes [31] bound on the outer surface of the thylakoids. Thus, it will be of interest to identify the physiological substrate of this kinase activity and establish whether it plays a role in energy distribution as well as State I–State II transition.

The fact that *Prochloron* thylakoid kinase activity is not light-activated, suggests that the LHC polypeptides might be continuously phosphorylated, irrespective of the light conditions. This would imply that LHC in this organism will be able to interact with Photosystem I and will be located mostly in the unstacked region of the thylakoids, which constitute the major fraction of this organism's photosynthetic membrane [7]. If this were the case, one would not expect to observe light-induced state transitions, as indeed reported in this work. This interpretation is in agreement with the data published by Giddings et al. [7]. These authors have investigated the ultrastructure of *Prochloron* thylakoids by the freeze-fracture technique, and have found the same classes of EF and PF particles as in thylakoids of *Chlamydomonas* or higher plants. However, a large proportion of the particle of the stacked EF was of the small size (7.5 nm) with a much larger number of 8.2 nm particles found on the unstacked PFface in *Prochloron* as compared with the eukaryotic chloroplast. These results are in agreement with the small size of the photosynthetic unit of *Prochloron* [6]. Possibly, the 7.5 nm particles of the EFs represent Photosystem II units without LHCII, whereas the 8.2 nm particles of the PFu might represent both the Photosystem I complex and LHCII. The partition of LHCII, when not linked to Photosystem II on the P fracture face, has been demonstrated before [33].

The permanent activation of the *Prochloron* thylakoid kinase might be the result of an evolutionary adaptation due to the light conditions where this organism lives. At 7–10 m below the sea surface, most of the red light (not less than 680 nm) is filtered out and Photosystem I receives constantly less energy than Photosystem II [34,35]. Under such conditions, the mechanism of light activation of the thylakoid protein kinase and modulation of energy distribution by phosphorylation and dephosphorylation of the LHC protein has little use. Even if this mechanism had already evolved in the ancestor of *Prochloron* (which might be common to the present day *Prochloron* and eukaryotic chloroplasts), it did not have a high selective value. On the other hand, mechanisms which ensure continuous excitation of Photosystem I by LHC absorbing at wavelengths less than 680 nm, will have evolutionary advantages.

The Red Sea didemnid *Diplosoma virens* harbouring *Prochloron* is found at water depths of 12–40 m, with a few colonies observed even at 86 m, while the Pacific Ocean isolates of this organism were collected in shallow waters (not more than 5 m) where some light of not less than 680 nm is present [6]. In such conditions, a mechanism that ensures energy distribution between the two photosystems will be useful. It will thus be of interest to test whether a light-activated thylakoid protein kinase is present in *Prochlorons* grown in shallow waters and whether in these *Prochlorons* State I–State II transitions will occur.

Acknowledgements

We wish to thank Dr. Ora Canaani and Dr. Shmuel Malkin for their help with the measurement of State I → State II transitions. The in situ action spectra were performed in collaboration with Dr. B.B. Jorgensen of Aarhus University, Denmark. This work was partially supported by a Schönbrunn award from the Authority for Research & Development of The Hebrew University of Jerusalem.

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