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Biochimica et Biophysica Acta xx (2005) xxx–xxx
BIOCHIMICA ET BIOPHYSICA ACTA
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Rapid report

Antenna ring around trimeric Photosystem I in chlorophyll *b* containing cyanobacterium *Prochlorothrix hollandica*

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Received 12 October 2004; received in revised form 7 February 2005; accepted 22 February 2005

Abstract

Prochlorothrix hollandica is one of the three known species of an unusual clade of cyanobacteria (formerly called "prochlorophytes") that contain chlorophyll *a* and *b* molecules bound to intrinsic light-harvesting antenna proteins. Here, we report the structural characterization of supramolecular complex consisting of Photosystem I (PSI) associated with the chlorophyll *a*/*b*-binding Pcb proteins. Electron microscopy and single particle image analysis of negatively stained preparations revealed that the Pcb–PSI supercomplex consists of a central trimeric PSI surrounded by a ring of 18 Pcb subunits. We conclude that the formation of the Pcb ring around trimeric PSI represents a mechanism for increasing the light-harvesting efficiency in chlorophyll *b*-containing cyanobacteria.

Keywords: Photosystem I; *Prochlorothrix hollandica*; *Prochlorococcus marinus*; Prochlorophyta; Chlorophyll *a*/*b* antenna protein; Electron microscopy

The freshwater phytoplankton *Prochlorothrix hollandica* is a filamentous cyanobacterium which was found in shallow eutrophic lakes in The Netherlands [1]. *Prochlorothrix*, as well as *Prochloron didemni* and *Prochlorococcus marinus*, species belong to a specific clade of cyanobacteria (formerly called "prochlorophytes") that do not collect the light radiation energy for photosynthesis like other cyanobacteria using water-soluble phycobilisomes, but instead they use intrinsic light-harvesting proteins that bind chlorophyll (Chl) *a* and *b* molecules or their analogs [2]. The Chl *a*/*b*-binding proteins (encoded by *pcb* genes) are predicted to have six transmembrane helices, and thus, they are not phylogenetically related to the *cab* genes encoding a superfamily of light-harvesting antenna proteins of green plastids [3]. Instead, they are similar to the iron-stress-induced *isiA* gene product of cyanobacteria and to the CP43 protein, a constitutively expressed Chl *a* inner antenna protein of Photosystem II [4]. *P. hollandica* has a cluster of three *pcb* (prochlorophyte chlorophyll *b*) genes which are organized in tandem and cotranscribed [5]. The major antenna polypeptides of 32 and 33 kDa are encoded by the *pcbA* and *pcbB* genes, respectively. The *pcbC* gene is significantly divergent from the other two genes and may have originated from a gene duplication independent of the one that led to *isiA* or the other prochlorophyte *pcb* genes [5].

Recent electron microscopic studies have shown that in iron-stressed conditions, the IsiA protein forms rings around the cyanobacterial trimeric Photosystem I (PSI) reaction center core complex [6–8]. A similar light-harvesting antenna ring has been discovered in a low light adapted strain of the prochlorophyte *P. marinus* SS120 [9]. In this case, the antenna ring contained 18 Pcb subunits forming the Pcb–PSI supercomplex and the presence of this antenna ring did not depend on iron deficiency. However, the Pcb–PSI supercomplexes have not been detected in other strains of prochlorophytes analyzed so far, the high-light genotype of *P. marinus* MED4 [10] and *P. didemni* [11]. In this work,

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0005-2728/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2005.02.005

we present the results of a structural study of *P. hollandica.* Our data complement the previous reports and support the role of Pcb antennae in the light harvesting of PSI in the prochlorophyte family where Pcb ring-like structures form a unique mechanism that significantly increases the size of the light-harvesting antenna system in unfavorable light-limiting conditions.

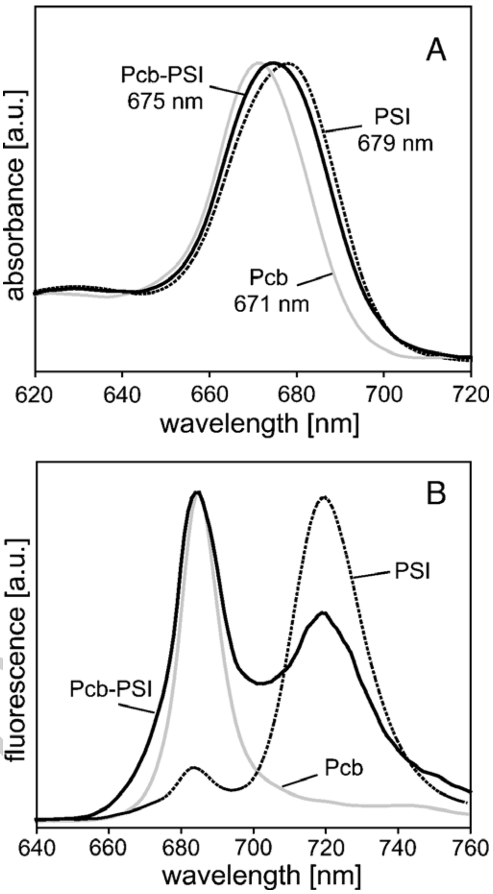

Fig. 2. Spectroscopic characterization of fractions derived from sucrose density centrifugation. (A) Room temperature absorption spectra. (B) Fluorescence emission spectra at 77 K excited by 430 nm. The Pcb–PSI supercomplex (black line), Pcb proteins (gray line) and PSI trimer (dotted line). Spectra were normalized at their peak maxima.

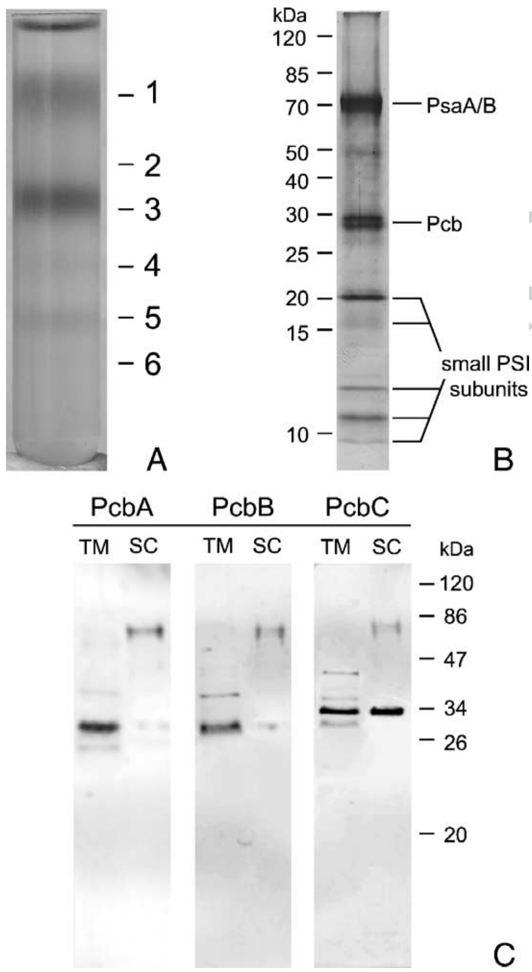

Fig. 1. Biochemical characterization of the Pcb–PSI supercomplex from *P. hollandica*. (A) SDS-PAGE analysis of the Pcb–PSI supercomplex. Proteins were separated on a 12–20% denaturating gradient gel and detected by silver staining. (B) Immunoblot analysis of Pcb proteins in the thylakoid membranes (TM) from *P. hollandica* and the Pcb–PSI supercomplex (SC), using polyclonal antisera raised against the PcbA, B and C proteins. All lanes contain 0.5 μg of chlorophyll.

The cells of *P. hollandica* were obtained from the Göttingen algal collection (SAG 10.89) and grown in BG11 medium under continuous illumination at an irradiance level of 15 μmol photons m^{-2} s^{-1}. Thylakoid membranes were prepared as in Ref. [12], but cells were broken with glass beads (100–200 μm in diameter) in a Beadbeater cell homogenizer (BioSpec Products, Inc.). The thylakoid membranes were solubilized with 1% *n*-dodecyl-β-D-maltoside (DM) and subjected to a sucrose density gradient centrifugation. The biochemical and spectroscopic characterization of the gradient was carried out as in Ref. [13,14]. For immunoblotting, proteins were separated by SDS-PAGE and transferred onto nitrocellulose and immunolabeled with polyclonal antisera raised against the synthetic oligopeptides of the PcbA protein L110-K125 (LKGPEDLSQSDFEFAK), PcbB protein R112-A127 (RFGPESIGEGSDSKFA), and the PcbC protein E2-D23 (EECSCDNRFRRGNNEPAGFSLD), respectively (Clonestar Biotech, Brno, Czech republic). The lowest green band of the sucrose gradient containing the Pcb–PSI supercomplex was applied on glow-discharged carbon coated grid and negatively stained with 2% uranyl acetate. Electron microscopy was performed with Philips TEM 420 electron microscope using 80 kV at 60,000× magnification. Micrographs were digitized with a pixel size corresponding to 0.45 nm at the specimen level. Image analyses were carried out using SPIDER software [15]. From 65 micrographs of the Pcb–PSI preparation, about 1500 top view projections were selected for analysis. The selected projections were rotationally and translationally aligned, and treated by multivariate statistical analysis in combination with classification as in

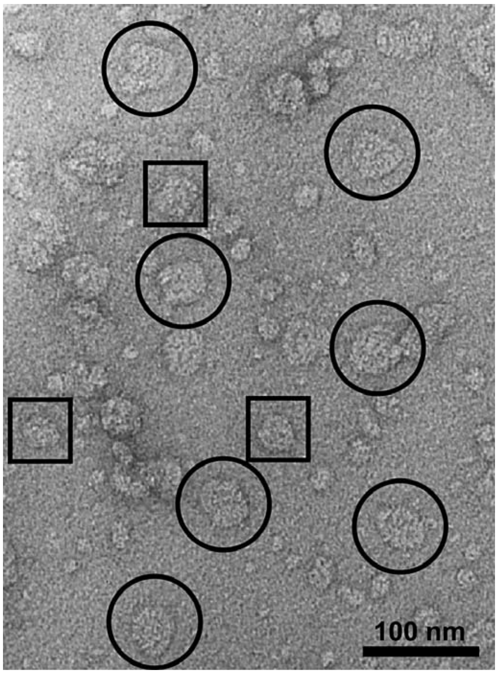

Fig. 3. Electron micrographs of the purified Pcb–PSI supercomplexes negatively stained with 2% uranyl acetate. Top view projections of the Pcb–PSI supercomplexes are circled, top view projections of the trimeric PSI are boxed. The bar represents 100 nm.

Ref. [13]. For molecular modeling, the coordinates were taken from Protein Data Bank (www.rcsb.org/pdb) under the code 1JB0 for PSI at 2.5 Å structure [16] and the code 1S5L for PSII at 3.5 Å structure [17].

Sucrose density gradient centrifugation of *P. hollandica* thylakoid membranes solubilized with DM resulted in the separation of six green bands (Fig. 1A). All bands were characterized by their protein composition, absorption and 77 K fluorescence emission spectra, and by size exclusion chromatography. These results clearly indicate that bands 1 and 2 contained Pcb antenna proteins and bands 3 and 5 corresponded to the monomeric and trimeric PSI complexes, respectively. The polypeptide composition of band 6 revealed major proteins of the PSI complex, such as the heterodimer of the PsaA/B reaction center proteins and several small PSI subunits with a molecular weight less than 20 kDa (Fig. 1B). Additionally, SDS-PAGE of band 6 resolved a band with apparent mass of about 30 kDa corresponding to Pcb proteins. Immunoblot analyses of the Pcb proteins in band 6 showed that only the PcbC protein was present (Fig. 1C). From these results we conclude that the lowest green band 6 of the sucrose density gradient is a Pcb–PSI supercomplex consisting of the PSI reaction center subunits associated with the PcbC protein.

Room temperature absorption spectrum of the Pcb–PSI supercomplex exhibited absorption maximum in the red region at 675 nm (Fig. 2A). The red peak was blue-shifted as compared to PSI trimers absorbing at 679 nm, due to the presence of the Pcb proteins, with absorption at 671 nm. The 77 K fluorescence emission spectrum of the supercomplex showed emission peaks with two maxima at 685 nm and 719 nm (Fig. 2B) corresponding to the energetically unbound Pcb proteins and to PSI, respectively. Since the fluorescence excitation spectra for the 719 nm emission peak of the Pcb–PSI supercomplex indicated efficient energy transfer to PSI (data not shown), we concluded that in this preparation, either a small portion of free Pcb subunits was presented as contaminants or the yield of energy transfer from Pcb subunits to PSI is not fully efficient.

Electron microscopy images of negatively stained preparations reveal a mixture of several types of particles (Fig. 3). The most abundant projections were circular particles with a diameter of about 34 nm that correspond to a Pcb–PSI supercomplex (Fig. 2, ringed). Smaller particles have diameter of about 22 nm (Fig. 3, boxed) and well resemble the PSI trimer [18]. The images were processed by single particle image analysis and the average projection map of the Pcb–PSI supercomplex is shown in Fig. 4A at a resolution of about 26 Å. The central region of

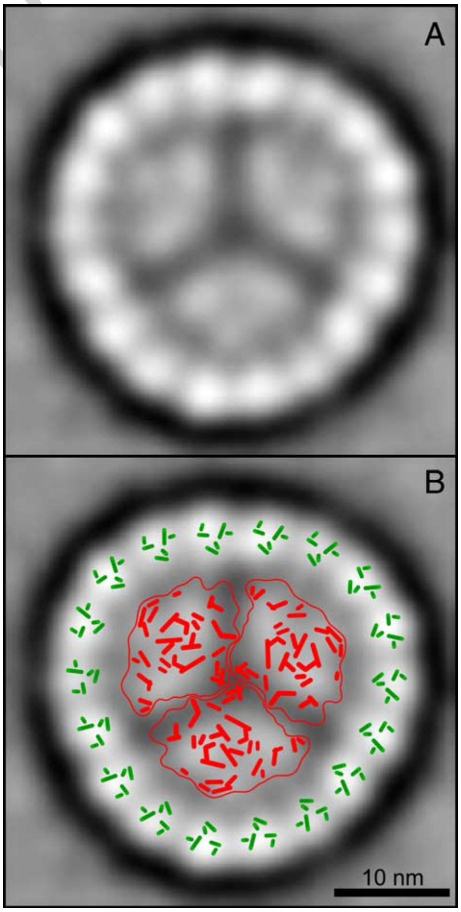

Fig. 4. The Pcb–PSI supercomplex isolated from *P. hollandica*. (A) The average top view projection map of the Pcb–PSI supercomplex with imposed three-fold rotational symmetry derived from an image analysis of 850 negatively stained particles viewed under electron microscopy. (B) Top view projection map of the Pcb–PSI supercomplex overlaid with the cyanobacterial X-ray model of the PSI trimer [14] and transmembrane helices of the CP43 protein of cyanobacterial PSII [15].

the particle has a three-fold symmetry and is surrounded by a ring of 18 separate densities. This structure is remarkably similar to that of the IsiA–PSI supercomplex isolated from two cyanobacterial strains, *Synechocystis* PCC 6803 [7] and *Synechococcus* PCC 7942 [8], both grown under iron-deficient condition, and to the Pcb–PSI supercomplex isolated from the low-light adapted strain *Prochlorococcus* SS120 [9]. Fig. 4B shows an incorporation of the X-ray structure of trimeric PSI complex and CP43 into the projection map of the Pcb–PSI supercomplex. This model suggests that the central region consists of PSI trimer surrounded by 18 subunits of the Pcb protein.

As shown in Fig. 1C, immunoblot analysis revealed the PcbA and PcbB proteins are not associated with the Pcb–PSI supercomplex whereas only the PcbC protein is present within the supercomplex. We suppose that in *P. hollandica*, there are specific Pcb antenna proteins for each photosystem: the PcbC protein for PSI and the PcbA and PcbB proteins for PSII. This result is in agreement with a recent study on Pcb antenna proteins in *Prochlorococcus* SS120, where the PcbC and PcbG subunits are targeted to PSI, and the remaining Pcb antenna proteins form constitutive PSII antennae [10]. In addition, phylogenetic analyses of the *pcb*/*isiA*/*psbB*/*psbC* gene superfamily indicate that the PcbC/G of *Prochlorococcus* SS120 and PcbC of *P. hollandica* form a separate cluster with regard to the other Pcb's from *Prochlorococcus* SS120 and PcbA and PcbB of *P. hollandica*, respectively [19].

The *P. hollandica* cells were originally collected from the Loosdrecht Lakes in The Netherlands [1]. Although the mean depth of the lakes is about 2 m, the water is so turbid that the euphotic layer is very shallow. To survive in these low light conditions, an additional antenna system of the type reported here increases the collecting capacity of PSI. Since the trimeric PSI binds about 300 chlorophyll molecules, the ring of Pcb proteins together with 270 chlorophylls increases the light-harvesting capacity of PSI by 90%, assuming that each of 18 Pcb subunits binds 15 chlorophyll molecules [5]. The PSI antenna rings were observed in *Prochlorococcus* SS120 [8] and *Prochlorothrix* (this study), both adapted to the very low irradiances either at the bottom of the euphotic zone or in shallow turbid waters. However, no Pcb rings have been observed in the high-light adapted strain *Prochlorococcus* MED4 [10] or in *P. didemni* [11] that grows symbiotically in shallow and clear tropical waters. We suggest that the formation of the Pcb rings around trimeric PSI represents a mechanism for increasing the light-harvesting efficiency of chlorophyll *b*-containing cyanobacteria.

Acknowledgments

We gratefully acknowledge the financial support of the Ministry of Education, Youth and Sports of the Czech Republic MSM6007665808 and of the Academy of Sciences of the Czech Republic AV0Z50510513 and AV0Z5020903.

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