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Purification and characterisation of photosystem I from the unusual cyanobacterium *Gloeobacter violaceus* - evidence for a membrane intrinsic light harvesting complex

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This article is dedicated to Prof. Wilhelm Menke on the occasion of his 91st birthday

Introduction

Gloeobacter violaceus (called *Gloeobacter* hereafter) is a cyanobacterium which, according to 16S rRNA data, diverges very early from the common cyanobacterial phylogenetic branch [Nelissen et al., 1995]. It is the only cyanobacterium which does not contain any thylakoid membranes [Rippka et al., 1974]. Instead, it contains a green plasma membrane carrying both, the photosynthetic and the respiratory electron transport chains. The photosynthetic apparatus of *Gloeobacter* has been shown to be extraordinary in more than one respect: It lacks the long wavelength chlorophyll fluorescence that all investigated cyanobacterial strains have been shown to emit at 77K [Koenig & Schmidt, 1995]. Furthermore, its phycoerythrin contains two different chromophores, phycoerythrobilin and phycourobilin, the latter of which is typical for rhodophytes and among cyanobacteria only found in few marine strains [Bryant et al., 1981]. Also, the membrane lipid composition is exceptional among photosynthetic organisms as it does not contain sulfoquinovosyl diacylglycerol (SQDG) [Selstam & Campbell, 1996]. The absence of the long wavelength fluorescence emission at 77K initiated the establishment of a purification procedure for PS I from *Gloeobacter*. The preparation shows some unusual properties, the most outstanding of which is a first evidence for the occurrence of LHC-like proteins in cyanobacteria on a protein basis.

Materials and methods

Gloeobacter SAG 7.82 (PCC 7421) was grown in Allen's medium [Allen, 1968] in continuous white light (4 µmol photons m^{-2} s^{-1}) at 21°C. Cells were harvested 6 months after inoculation. A crude membrane suspension was produced by breaking the cells with a glass bead homogeniser and subsequently centrifuging the homogenate to dispose of cellular debris. The resulting supernatant was complemented with dodecyl-*ß*-D-maltoside (*ß*-DM) to a final concentration of 1.2% (w/v). Typically, the molar Chl : *ß*-DM ratio was 1 : 2500. The suspension was stirred in the dark at 4°C for 30 min, then centrifuged for 15 min at 150,000 ×*g*, 4°C. The supernatant was filtered (45 µm) and immediately loaded onto a Poros HQ50 column (Applied Biosystems) equilibrated with low salt buffer (20 mM Tris/HCl, pH 8.0,

10 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM NaCl) supplemented with 0.3% *ß*-DM. Elution from the column was achieved with a linear gradient of NaCl up to 300 mM at a flow rate of 6.75 ml min^{-1}. Chlorophyll containing fractions were loaded onto a Superose 6 gel filtration column (Pharmacia) equilibrated with 20mM Tris/HCl, pH 8.0, 10mM $MgCl_2$, 5 mM $CaCl_2$, 150 mM NaCl, 0.03% (w/v) *ß*-DM at a flow rate of 0.5 ml min^{-1}. The column was calibrated with pure preparations of monomeric (300 kDa) and trimeric (800 kDa) PS I from *Spirulina platensis*.

Electrophoretical protein subunit analysis was done with the Tris/Tricine system [Schägger & Jagow, 1987]. For immunodetection proteins were transferred to a PVDF membrane (Immobilon, Millipore). Antibodies against PS I subunits from spinach were provided by R. Berzborn (Bochum, Germany), antibodies against the major LHC from *Cyclotella cryptica* were donated by E. Rhiel (Oldenburg, Germany). 77 K fluorescence spectra were recorded with an Aminco Bowman luminescence spectrometer. Chlorophyll concentration was adjusted to 1µg ml^{-1} in 50% glycerol. Flash-induced absorption changes [Hiyama & Ke, 1972] were measured between 650 and 750 nm at a chlorophyll concentration of 3.45 µM in a buffer containing 50 mM ACES, pH 6.5, 50 mM KCl, 30 µM PMS and 5 mM sodium ascorbate. Transmission electron microscopy was performed as described before [Lelong et al., 1996] by diluting the samples 5x with NaCl-free buffer plus 0.03% *ß*-DM. Image analysis was carried out with IMAGIC software [van Heel, 1989]. The projections were analysed as described by Harauz and coworkers [1989] and treatment by multivariate statistical analysis [van Heel & Frank, 1981] and classification [van Heel, 1989].

Results and discussion

PS I from *Gloeobacter* was purified by anion exchange chromatography (data not shown). The preparation contained monomers as well as trimers. The apparent molecular weight of the trimers was determined by gel filtration to be 1100 kDa (data not shown), which is significantly higher than the value of previously isolated PS I trimers from other cyanobacteria, *Spirulina platensis* and *Synechocystis* PCC 6803 (800 kDa). The most plausible explanation for this might be that PS I specifically forms complexes with other proteins present in the preparation.

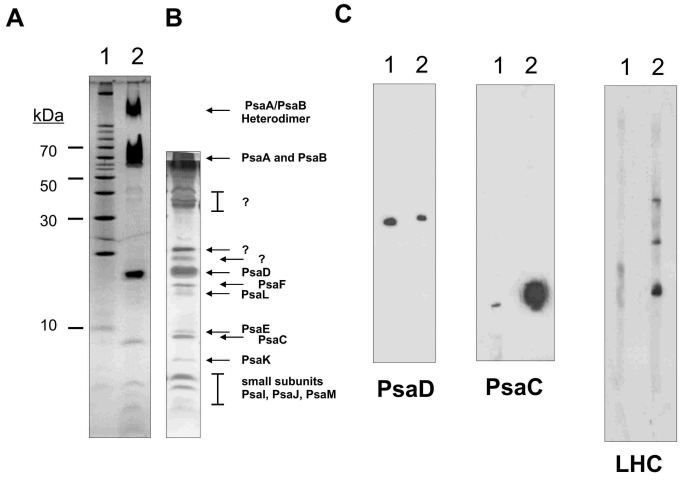

Fig. 1: Tris/Tricine PAGE analysis of the purified PS I fraction. (A) Coomassie Blue stained and (B) silver stained gels are shown. Identified subunits are shown in the picture. (C) Immunological identification of the PsaD and PsaC subunits using antisera raised against spinach proteins, and positive reaction of *Gloeobacter* PS I subunits with antibodies against an LHC protein of *Cyclotella cryptica* (diatom). Lane 1 represents *Synechocystis*, Lane 2 *Gloeobacter*.

The subunit composition of the purified complexes shows a typical PS I pattern in Tris/Tricine PAGE [Kruip et al., 1993], besides some additional bands, which could not be attributed to known proteins (Fig. 1). Four of the PS I subunits were

unambiguously identified by detection with specific antibodies against PsaD, PsaC (Fig. 1C), PsaF and PsaL (data not shown).

The sample was probed with an anti-serum against the major LHC from the diatom *Cyclotella cryptica* (Fig. 1C) [Rhiel et al., 1997]. Clearly, three immunologically related proteins were detected in *Gloeobacter* whereas no cross-reaction could be shown for *Synechocystis*. The respective polypeptides from *Gloeobacter* have apparent molecular masses of about 9, 15 and 23 kDa. The results presented here possibly constitute a first evidence for three helix LHCs in cyanobacteria, implying that LHC proteins were already invented in the prokaryotic ancestor of chloroplasts rather than upon evolution of the eukaryotic cell as discussed recently [Green, 2001].

The difference spectrum [Hiyama & Ke, 1972] of purified *Gloeobacter* PS I has the shape of a typical P700 difference spectrum (Fig. 2). From the amplitude of the *Gloeobacter* spectrum at 700 nm a Chl : P700 ratio of 147 is obtained with an extinction coefficient of 64 mM^{-1} cm^{-1}. Since normally PS I is known to contain 90 to 100 chlorophylls [Schubert et al., 1997], it is not likely that *Gloeobacter* PS I should be able to accommodate around 50 % more chlorophylls without possessing additional, chlorophyll bearing subunits. Indeed, novel PS I associated proteins are present in high amounts in the preparation described here (Fig. 1A and B).

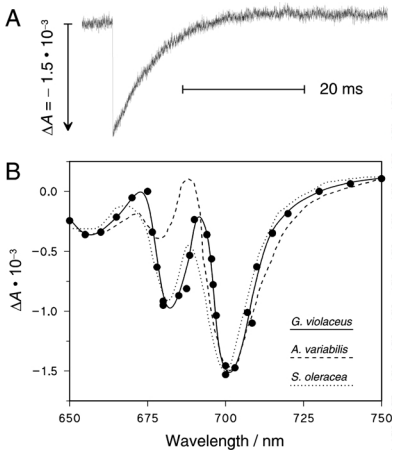

Fig. 2: Characterisation of purified PS I complexes from *Gloeobacter* by flash induced absorption changes. Signals from 64 flashes (given at a frequency of 1 s^{-1}) were averaged for each measured wavelength. Each sample was used at four different wavelengths, no ageing effects were observed during this procedure and sample stability allowed for several hundred flashes without detectable loss of signal amplitude. **A** Time course at 700 nm. The half time for signal relaxation at 700 nm is 5 ± 1 ms. **B** Difference spectrum.

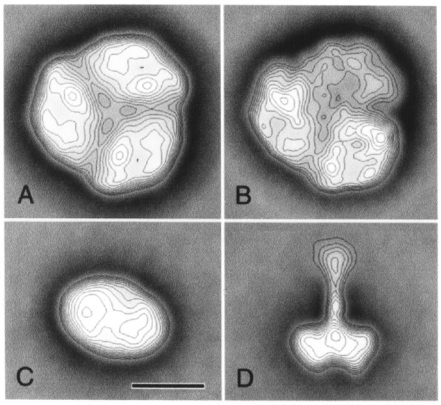

Fig. 3: Statistical analysis of electron micrographs of purified complexes contoured with equidistant contour lines. **A** and **B** Sum of 300 / 362 PS I trimers. **C** Sum of 471 PS I monomers. **D** Sum of 406 T-shaped complexes. The scale bar represents 10 nm.

The isolated PS I particles are physiologically very stable: Even over a time span of 36 hours at room temperature, no decay in signal intensity could be found.
77 K fluorescence spectroscopy confirms that the typical cyanobacterial long wavelength emission is absent in *Gloeobacter*, also in purified PS I complexes (data not shown) [Koenig & Schmidt]. The spectrum resembles the 77K fluorescence emission spectrum of PS I from cyanobacteria grown under iron limitation [Boekema et al., 2001].

Electron microscopy of the purified *Gloeobacter* PS I fraction reveals mainly typical PS I trimers (Fig. 3A and B) with a minority of PS I being present as monomers (Fig. 3C). Both monomers and trimers are remarkably similar to previously analysed PS I particles from other species regarding size and shape [Kruip et al., 1997], despite the significantly higher molecular mass as determined by gel filtration. Interestingly, an additional complex of very unusual structure is visible in the preparation consisting of a bilobed scaffold with a width of 13 nm and a height of 6.5 nm from which a long (12 nm) and extremely narrow (2.5 nm) stem protrudes almost vertically (Fig. 3D). Similar complexes have recently been observed in PS I trimer preparations isolated from *Synechococcus* PCC 7942 grown under iron limitation [Boekema et al., 2001]. These complexes might be suggested to specifically interact with PS I trimers, thus accounting for their higher molecular weight.

Summary

A purification protocol which yields trimeric PS I from *Gloeobacter violaceus* has been established. The trimeric complex has the same subunit composition and dimensions as PS I from other cyanobacteria. Nevertheless the preparation shows some unexpected properties: A high Chl : P700 ratio, an atypical 77K fluorescence emission spectrum and some co-purifying proteins which might represent LHC-related proteins

and could be identical with the T-shaped complexes seen in electron micrographs. *Gloeobacter* is a key-stone organism for understanding the evolution of complex membrane systems and the evolution of membrane based energy generating systems. In this study some unusual features of PS I from *Gloeobacter* have been presented which form the basis of a molecular understanding of membrane organisation and energy metabolism in this organism. Most striking is the first evidence of LHC-like proteins in a cyanobacterium. This finding is thought to have general implications for the evolution of chloroplasts.

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