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Characterization of purified His-tagged CP47-containing photosystem II complexes from a cyanobacterium, *Synechocystis* sp. PCC 6803

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Introduction

The PS II complex consists of more than 20 polypeptides including several small polypeptides, the molecular masses of which are below 10 kDa [Funk 2000]. Although Zouni et al. [2001] reported the crystallographic structure of 3.8Å resolution, the whole polypeptide components are not yet clear from their data. There still is a possibility that the PS II complex has some unknown polypeptides of low molecular masses. For the determination of the precise structure of PS II complexes, all the polypeptide constituents must be identified.

Bricker et al. [1998] introduced His-tagging on the C-terminus of the *psbB* gene product, CP47, and successfully purified PS II reaction center complexes from the mutant of mesophilic cyanobacterium, *Synechocystis* sp. PCC6803 (HT-3) with a high activity within one day. Because of the simple and mild purification procedure, it is reasonably expected that the purified PS II complexes retain all the constituents. Recently, we have improved a SDS-PAGE method, which can resolve polypeptides in a wide molecular mass region, from lower than 3 kDa to over 100 kDa [Kashino 2001]. The recent advances of a proteomics technique with mass-spectrometry are also beneficial to identify polypeptides even when their N-terminal sequences are not available. Furthermore, the whole genome sequence database of *Synechocystis* 6803 is now available [www.kazusa.or.jp/cyano/cyano.html]. Using these methods, here, we tried to identify the full member of the PS II complexes in *Synechocystis* 6803.

Materials and methods

Cell culture and PS II purification - A HT-3 mutant of *Synechocystis* 6803 was cultivated in BG11 medium supplemented with 5 mM glucose and 50 μM kanamycin in a cylindrical 3 L bottle under 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ at 30°C with air bubbling for 3 days. The PS II complexes were purified as described in [Bricker 1998].

SDS-PAGE - Polypeptides were resolved according to [Kashino 2001].

N-terminal amino-acid sequencing - N-terminal sequences of polypeptides were analyzed after [Kashino 2001], using a protein sequencer (model 473A, Applied Biosystems). The polypeptides were identified using the database, CyanoBase.

MALDI mass spectrometry - Each polypeptide separated and stained on SDS-PAGE gel was excised, and subjected to in-gel digestion with trypsin. The extracted peptides were analyzed by matrix assisted laser desorption (MALDI) mass spectrometry. MALDI was performed on a DE-STR instrument (PerSeptive Biosystems, CA) using α-cyano-4-hydroxycinnamic acid as the matrix. Tandem mass spectrometry was performed using nanoLC/MS/MS. The Protein Prospector suite of programs [http://prospector.ucsf.edu/] was used for searching the CyanoBase.

Oxygen-evolving activity - Oxygen evolution was measured using a Clark-type oxygen electrode at 30°C in a buffer containing 50 mM MES-NaOH (pH6.0), 10 mM $MgCl_2$, 5 mM $CaCl_2$, 5 mM NaCl and 0.5 M sucrose using 0.5 mM 2,5-dichloro-*p*-benzoquinone (DCBQ) and 1 mM ferricyanide as electron acceptors. The Chl concentration was 2 μg Chl *a*·mL^{-1}.

Results

Figure 1 shows a polypeptide profile of purified PS II complexes. The fluorescence spectra at 77K also revealed the total removal of phycobiliproteins (around 16 kDa) and PS I complexes (data not shown). It is clear that the PS II complex was highly but easily purified by the His-tagging technique [Bricker 1998]. Purified PS II complexes showed a single peak at around 450 kDa on size exclusion chromatography (data not shown), suggesting the purified HT-3 PS II complexes were in a dimeric form. In the purified PS II complexes, more than 30 polypeptide bands were recognized. All the polypeptides were applied to N-terminal sequencing and to MALDI peptide mass mapping. In addition to the well-known major polypeptides, the PS II complexes had several low-molecular-mass polypeptides of less than 7 kDa, those of which were PsbH, PsbL, PsbI, PsbT, PsbJ, PsbX, PsbM, PsbK and PsbY. PsbN was not found. The purified PS II complexes had also several polypeptides which had not been recognized as members of PS II until now; these were the products of *sll1281* (*ycf9*, about 5 kDa), *sll1130* (about 13 kDa) and *sll1414* (about 25kDa) genes. Furthermore, MALDI peptide mass mapping revealed the presence of FtsH and a polypeptide coded by *sll1638*, whose N-termini were blocked.

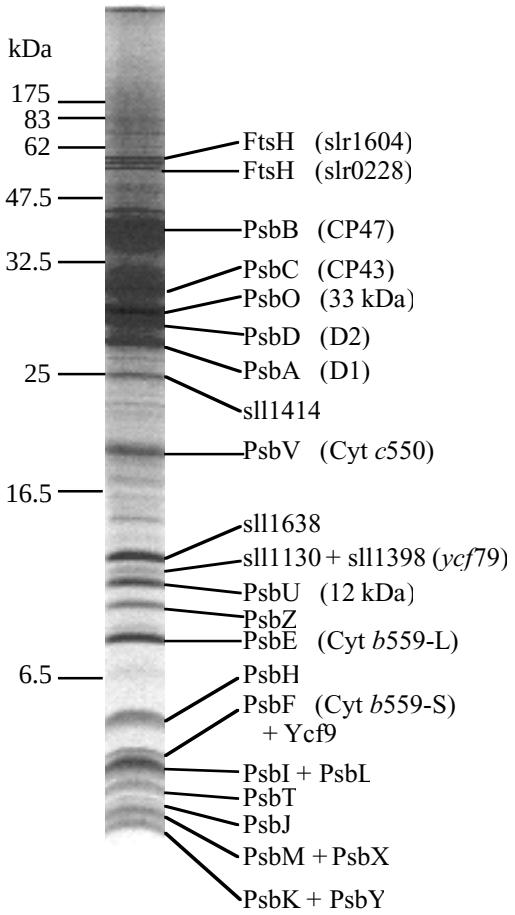

Fig. 1. A polypeptide profile of purified His-tagged PS II complexes from the HT3 strain of *Synechocystis* sp. PCC 6803. The positions of molecular-weight standards are shown on the left. A 10 μg Chl *a*-containing sample was applied to the electrophoresis, and the polypeptides were stained with Coomassie blue.

The purified PS II complex contained 41.0 Chl *a* molecules per C550 (i.e. per reaction center of PS II). It also contained 1.0 Ca, 5.2 Fe and 3.8 Mn atoms (atomic absorption spectroscopy analysis), 2.4 molecules of pheophytin *a*, 11 molecules of β-carotene, 1.3 molecules of zeaxanthin (HPLC analysis) per 41 Chl *a* molecules. The amount of cytochrome b_{559} was 1.9 per 41 Chl *a* molecules (differential absorption spectroscopy [Cramer 1986]).

The pool size of electrons in the absence of DCMU was 3.3 times larger than that in the presence of DCMU (fluorescence induction kinetics).

The purified PS II complexes evolved oxygen at a rate as high as 2,610 μmoles O_2·mg^{-1} Chl a·hr^{-1} in the presence of 0.5 mM DCBQ and 1 mM ferricyanide as electron acceptors. For the high activity of oxygen evolution, Ca^{2+} was necessary. The stability of this high oxygen-evolving activity was assessed (Figure 2). After 16 days of incubation at 4°C in the dark, the purified PS II complexes retained 80% of the initial activity. Although the activity decreased with longer incubation, about a half of the initial activity was remained after 34 days of incubation.

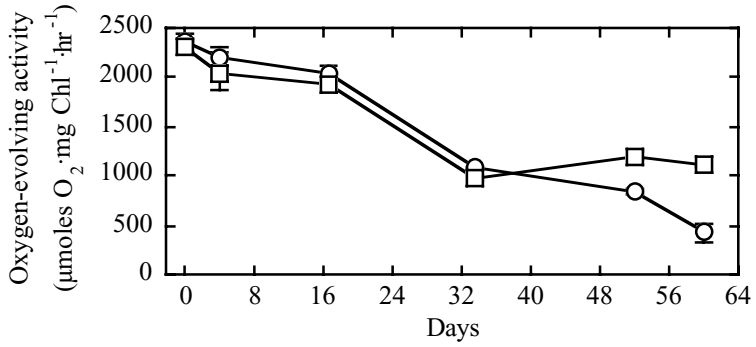

Fig. 2. Stability of the purified PS II complexes. After incubation of the PS II complexes at 4°C in the dark for various days, the oxygen-evolving activity was measured at 30°C.

Discussion

Using the HT-3 mutant of *Synechocystis* 6803 [Bricker 1998], we purified highly active and interesting PS II complexes. The oxygen-evolving activity reached 2,600 μmoles O_2·mg Chl a^{-1}·hr^{-1}. It was very stable when the complex was incubated at 4°C in the dark (Figure 2). The purified PS II complexes had no contamination of phycobiliproteins and PS I complexes. Although the requirement for Ca^{2+} of the oxygen-evolving activity and the large antenna size determined by oxygen yield (86 Chl *a* per reaction center of PS II) suggest some impairment on the oxidizing side of PS II, the purified PS II complexes seem to be suitable to determine the whole constituents of PS II, including metals and low-molecular-mass components.

All the Coomassie-stainable polypeptides were identified by use of the recently introduced SDS-PAGE system in conjunction with the N-terminal sequencing and the MALDI mass spectrometry. Other than well-known major polypeptides of PS II, several polypeptides, which had not been reported as PS II components or which had not been reported in the cyanobacterial PS II complexes, were found. PsbY [Meetam 1999] and PsbJ [Lind 1993] are included in the latter case. The former includes Ycf9, the *sll1638* gene product and FtsH.

Ycf9 was also identified in our purified PS II complexes. Recently, Mäenpää et al. [2000] showed the presence of Ycf9 in the stromal thylakoid fraction in higher plants by immunoblotting, but not in the granal region. We propose that Ycf9 plays an important role in an early stage of the assembly of PS II complexes.

The *sll1638* gene product was first identified in this work. The deduced amino acid sequence has a high homology to the PsbQ protein, the extrinsic 17 kDa protein of PS II in higher plants and green algae. The homology of latter half of the *sll1638* gene product was 51% against the PsbQ protein of *Arabidopsis thaliana* and 46% against that of *Chlamydomonas reihardtii*. Quite different from PsbQ, it has one putative trans-membrane

region in the N-terminus and could not be removed from the PS II complexes by Ca^{2+}-washing (data not shown). It had been believed that the cyanobacterial PS II complex does not possess a protein(s) homologous to PsbQ. It is highly possible that the *sll1638* gene product is the origin of PsbQ. Deletion mutant of this gene is already constructed, and it will be reported elsewhere.

The FtsH protein is one of the distinct components. Lindahl et al. [2000] showed that the protein catalyzes the degradation reaction of the 23 kDa fragment of the D1 protein in *Arabidopsis thaliana*. Our data clearly showed the presence of FtsH in the PS II complex, further supporting that FtsH functions in PS II. This result, in turn, also demonstrates that the HT-3 PS II complex was purified in a highly intact form.

The presence of the PsbN protein was not confirmed. Ikeuchi et al. [1995] also failed to detect the PsbN protein in isolated PS II complexes from *Synechocystis* 6803. Recently, we found that the polypeptide which was originally assigned as PsbN was PsbT, which will be reported elsewhere.

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