Isolation and Characterization of Photosystem II Complex from Red Alga Porphyra haitanensis

Ying Xia Li1,2, Guang Ce Wang2*, Jian Feng Niu2, Zheng Quan Gao3 & Chang Sheng Chen5,4

1School of Life Science and Technology, Nanyang Normal University, Nanyang 473 061, China
2Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, Qingdao 266 071, China.
3Nation Deep Sea Center of State Oceanic Administration, People’s Republic of China, Qingdao, China
4School of Life Science, Shandong University of Technology, Zibo, China
5Institute of Fishery Biotechnology, Department of Aquaculture, Jimei University, Xiamen 361 021, China

[E-mail: liyx108@163.com]

Received 22 March 2011; revised 3 October 2011

Thylakoid membranes were isolated and purified from gametophyte of Porphyra haitanensis by sucrose density gradient ultracentrifugation. After P. haitanensis gametophyte thylakoid membranes were solubilized with sodium dodecyl sulfate (SDS), the highly pure and active photosystem (PS) II complex was isolated and characterized by the room absorption and fluorescence emission spectra, DCIP (2, 6-Dichloroindophenol) reduction and oxygen- evolving rates. The PS II complex from P. haitanensis had the characteristic absorption peaks of chlorophyll (Chl) a (436 and 676 nm) and typical fluorescence emission peak at 685 nm (Ex = 436 nm). Acquired PS II complex exhibited high oxygen evolution (879 µmol O2. mg-1 Chl. h-1) in the present of 2.5 mM DCIP as an artificial acceptor and was active in photoreduction of DCIP (2, 6-dichloroindophenol) by DPC (1, 5-diphenylcarbazide) at 104 ueq. mg-1 Chl. a. h-1. Extrinsic proteins of P. haitanensis PS II consisted of 33, 20, 16, 12 kD proteins and cyt c-550 (15 kD), and were different with that of cyanobacteria and higher plants.

[Keywords: Porphyra haitanensis, thylakoid membranes, photosystem II]

Introduction

Chloroplasts of red algae are descended from cyanobacteria, and retain many features of the cyanobacterial photosynthetic apparatus1. However, the structural organization of photosynthetic membranes, as well as the arrangement of photosynthetic units in thylakoids, differs significantly with cyanobacteria and higher plants2,3. Thylakoid membranes of red algae are not stacked as found in higher plants and green algae but lie equidistantly and singly within chloroplasts4,5. Both cyanobacteria and the red algae contain phycobilisomes that serve as the primary light-harvesting system instead of chlorophyll a/b (or chlorophyll a/c)-binding proteins existed in higher plants and other algae6,7,8. While red algae contain intrinsic chlorophyll-based light-harvesting complex (LHC) associated with photosystem I (PSI) like all photosynthetic eukaryotes9. Therefore, photosynthetic apparatus of red algae represent a transitional state between cyanobacteria and photosynthetic eukaryotes.

The PS II complex is a important pigment protein complex, which catalyzes the oxidation of water and the reduction of plastoquinone by utilizing photon energy10. The PSII is a multisubunit pigment protein complex including extrinsic and intrinsic components located in thylakoid membranes11. In green algae and higher plants, the extrinsic domain of the intact PSαcomplex of is composed of 33 kD, 23 kD and 17 kD proteins. While in cyanobacteria and red algae 23 kD and 17 kD proteins are replaced by cyt (cytochrome) c-550 and a 12 kD protein and red algae. The composition of red alga PSαcomplex is relatively complex and changed according to different red algae12.

Porphyra is a marine red alga genus that maintains several primitive features such as asexual reproduction through generation of monospores13. Porphyra haitanensis Chang et Zheng, an intertidal red alga with high economic value, only habits and widely cultured in south coastal region of China14. In order to understand photosynthetic apparatus of red algae, PS II were isolated from red alga P. haitanensis and compared with those from cyanobacteria and higher plant.
Material and Methods

The gametophytic blade of *P. haitanesis* Chang et Zheng was collected from the seashore of Xiamen, China. The alga was washed with sterile seawater to remove macroscopic contamination, then, dipped in distilled water for 10 min.

500 g of alga was fragmented in a triturator containing 2500 ml cold extract buffer (50 mM Tris, 5 mM EDTA, 1 mM MnCl₂, 1 mM MgCl₂, 2 mM NaNO₃, 100 mM Sucrose, 0.5 mM K₂HPO₄, pH 7.8) and further broken with ultrasonic at 4°C for 1 h (60 W, 6 s interval). Homogenate was filtrated through 8 layers of gauze and the filtrate was centrifuged at 5000 g for 5 min to remove large debris. The supernatant was collected and centrifuged at 140,000 g (Beckman L8-80, Ti-45 rotor) for 1 h at 4°C. Resulting pellet was suspended in cold extract buffer without sucrose, then, loaded on sucrose density gradient consisting of 60%, 50%, 40%, 30%, 20% (w/v) sucrose in proportions of 1:1:1:1:1. Sample was centrifuged at 140,000 g (Beckman L8-80, Sw-40 rotor) for 3.5 h at 4°C. Each band with different colors was dialyzed against the cold extract buffer without sucrose at 4°C for 24 h to remove sucrose.

The purified thylakoid membrane from *P. haitanensis* gametophytic blade was treated by 25 SDS to 1 Chl a (w/w) in the dark with gently stirring at 4°C for 30 min. Resultant mixture was loaded onto the sucrose density gradient consisting of 60%, 50%, 40%, 30%, 20%, 15%, 10% (w/v) sucrose in proportions of 1:1:1:1:1:1:1 containing 0.2% SDS, and ultracentrifuged at 140,000 g (Beckman L8-80, Sw-40 rotor) for 15 h at 4°C. Bands with different colors were dialyzed in the dark at 4°C for 24 h to remove sucrose.

\[ \text{Oxygen evolution rates of the samples were measured using a Clark-type electrode (Hansatech Instruments Ltd., England) with 20 \mu mol photons m}^{-2} s}^{-1} \text{ illumination at 17°C. Samples at a Chl a concentration of 10 ug Chl a mL}^{-1} \text{ were suspended in} \]

1.5 mL medium containing 20 mM Mes (pH 6.5), 0.3M sucrose, 20 mM CaCl₂, 10 mM NaHCO₃, 10mM NaCl, supplemented with electron acceptors, 2, 5-dichloro-benzoquinone at a concentration of 500 \mu M and ferricyanide at a concentration of 2.5 mM.

DCIP (2, 6-dichloroindophenol) photoreduction rates of every band obtained from the sucrose density gradient ultracentrifugation, either with or without added artificial electron donor DPC (1, 5-diphenylcarbazide), were measured spectrophotometrically at 580 nm (12.9 mM⁻¹ cm⁻¹), in a medium containing 40 uM DCIP and 30 mM MES- NaOH (pH 6.8). The concentration of samples was equivalent to 10 ug Chl mL⁻¹.

Samples were first precipitated with 9 volume of cold 90% acetone at -20°C for 1.5 h, then, centrifuged at 5000 g for 10 min. Pellet was suspended with loading buffer (0.25 M Tris, 5% glycerol, 1% SDS, 0.025% 2-Mercaptoethanol). Sample was applied to the gel with prior heating in boiling water for 5 min. Separating gel was 15% (pH 8.8), and the stacking gel was 5% (pH 6.8). Samples were separated using a constant voltage of 60 V at room temperature and visualized by staining with AgNO₃. The following proteins were used as molecular mass markers: phosphorylase b (97.4 kD), albumin (66.2 kD), ovalbumin (43.0 kD), carbonic anhydrase (31 kD), trypsin inhibitor (20.1 kD) and α-lactalbumin (14.4 kD).

Results

AS part of Isolation of thylakoid membranes, after the first sucrose density gradient ultracentrifugation, three main bands with different colors were presented in centrifuge tubes (Fig. 1). They were designated A1, F

Fig. 1—Results of the first sucrose density gradient ultracentrifugation.
A2 and A3 from the upper, which located in 0-20%, 40-50% and 50-60% sucrose layer, respectively.

Fig. 2 showed the visible absorption spectra of A1. Seven bands located at 436, 498, 545, 565, 614, 650 and 676 nm were detected in A1. Three peaks at 498, 545 and 565 nm were attributed to R-PE, and the other four peaks assigned to PC (614 nm), APC (650 nm) and Chl $a$ (436 and 676 nm), respectively, indicating that A1 was free pigment. A2 and A3 showed similar absorption peaks and consisted of pheophytin (419 nm), Chl $a$ (436 and 476 nm) and carotene (485 nm).

Isolation and characterization PS II consists the following. When A2 and A3 were solubilised gently with 25 (SDS) to 1 (Chl $a$) and ultracentrifugation, many bands with different colors were demonstrated (Fig. 3). Two bands, which located in 0-20% and in 40-50% sucrose layer, respectively, were designated as A2-a and A2-b were acquired from A2. Four main bands marked as A3-a, A3-b, A3-c, A3-d and A3-e were obtained from A3. A3-a located in 0-10% sucrose layer and A3-b was dark green in 15-20% sucrose layer. A3-c, A3-d and A3-e located in 30%, 30-40% and 40% sucrose layer, respectively.

Photochemical activities of DCIP reduction with DPC and $O_2$ evolution rates of every band acquired from the first and the second sucrose density ultracentrifugation, calculated on a Chl $a$ basis, were illustrated in Table 1. A3 evolved oxygen at a rate of 14 umol $O_2$. mg$^{-1}$ Chl. h$^{-1}$, and the second ultracentrifuged fragment A3-e exhibited a higher activity of 879 umol $O_2$. mg$^{-1}$ Chl. h$^{-1}$. A3 was found to be active in photoreduction of DCIP by DPC at 5.7 ueq. mg$^{-1}$ Chl. h$^{-1}$. This activity was lower than the 108.4 ueq. mg$^{-1}$ Chl. h$^{-1}$ of A3-d. Oxygen evolution and photoreduction activities could not be detected in the bands from A2, A2-a, A2-b and other four bands from A3. These results showed that A3-e was PSII particle and A3 was thylakoid membrane consisting of PSII particle.

The Fig. 4 displayed the room temperature absorption spectra and fluorescence emission spectra (Ex= 436 nm) of the purified PS II reaction center complex A3-e. Absorption spectra showed that the purified PS II particles contained pheophytin (420 nm), Chl $a$ (436 and 676 nm) and carotene (485 nm).

The polypeptide analysis of the purified PS II particles from the gametophytic blade of P. haitanesis were shown in Fig. 5. The purified PS II core complex consisted of 10 protein bands in
the SDS-PAGE gel, located at 47, 43, 38, 33, 31, 29, 20, 16, 15 and 12 kD.

Discussion

Although red algae are eukaryotic algae, their photosystems share many aspects with that of cyanobacteria. Consistent with this, PSII from the red algae are partially functional in both the cyanobacterial and higher plant. Understanding the structure and components of photosystems of red algae is significative in evolutionary biology, and isolation of pure oxygen-evolving PS II core complex is a crucial prerequisite. In order to isolate pure PS II core complex, many detergents were used to fragment chloroplasts. Although mild non-ionic detergent is better than ionic detergent because ionic detergent may lead to the loss of activity of PS II. However, PSII particle of *P. haitanesis* with high O$_2$ evolution rate and DCIP reduction activity could be isolated after solubilization with SDS. Similar result was observed in *P. yezoensis* PS II. So, *Porphyra* might have the nature resistance to detergent, especially especially to non-ionic detergent.

PS II of different algae showed different fluorescence emission peak. The PS II of *P. haitanesis* also only had a emission peak at 685 nm when excited at 436 nm. Similar result was consistent with the PS II of *Bangia fusco-purpurea, P. yezoensis*, and *Laminaria saccharina*, which only had a emission peak at 685 nm and 687 nm, respectively. While the PS II reaction center of *Spinacia oleracea, Pisum sativum, Cyanophora paradoxa* and *Porphyridium cruentum* had two fluorescence emission peaks, a main peak at about 685 nm and a shoulder around 695 nm.

In this study, the bands located at 47, 43, 31 and 29 kD were CP47, CP43 apoprotein, D2 and D1 proteins, respectively. These intrinsic proteins existed in all photosynthetic organisms, while extrinsic proteins in cyanobacteria, red algae and higher plants were different. 33, 23 and 17 kD proteins were existed in green algal and higher plant PS II. Whereas, cyanobacterial PS II contained two different extrinsic proteins, cyt c-550 and a 12 kD protein in addition to 33 kD protein. Previous studies showed that the extrinsic proteins varied in different red algae. Four extrinsic proteins (33, 20, 12 kD proteins and cyt c-550) were identified in PS II of two unicellular red algae *Cyanidium caldarium* and *Porphyridium cruentum*. Of these proteins, only 33 and 20 kD proteins and cyt c-550 were found in *P. yezoensis* PS II, but 12 kD protein was absent. In contrast, *P. yezoensis* PS II had two different extrinsic proteins, 16 and 14 kD proteins. In *B. fusco-purpurea* PS II four extrinsic proteins (33 kD protein, 20 kD protein, cyt c-550 and 16 kD protein) were found. In this study, the PS II of *P. haitanesis* contained five extrinsic proteins including cyt c-550, 33, 20, 16 and 12 kD proteins. Above results suggested that the extrinsic proteins of PS II differed somewhat between different red algae although 33, 20 kD and cytc-550 existed in these five red algae, and 20 kD protein was characteric protein in red algae.
Acknowledgement

Present work was supported by Project for Supporting the National Development (No. 2006BAD09A04) and the 863 Project of China (Nos. No. 2006AA10A413, 2006AA05Z112). The financial support is from the National Natural Science Foundation of China (Nos. U0633006, 40476059).

References