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Investigation of the *in vivo* structure of the oxygen-evolving photosystem II (PS II) core complex in *Synechococcus elongatus*

M SUGIURA and M TAKAHASHI

Department of Applied Biological Chemistry, Osaka Prefecture University, 1-1, Gakuencho, Sakai, Osaka, 599-8531, Japan. Phone, +81-72-254-9451; Fax, +81-72-254-9451; E-mail, miwa@biochem.osakafu-u.ac.jp

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Introduction

Oxygen evolution is a result of concerted redox reactions in PS II core complex having well-organized multisubunit structure. Assuming that eleven subunit molecules associate to form PS II core complex on the basis of 1:1 stoichiometry (Ikeuchi and Inoue 1988), the mass for the entire molecule could be estimated to be about 230 kDa. Corresponding size for PS II core complexes has been obtained from the size- and shape-analyses of the detergent-solubilized PS II complexes. We purified oxygen-evolving PS II core complexes from the cells of *Synechococcus elongatus,* a thermophilic cyanobacterium (Sugiura and Inoue 1999). The complexes held active oxygen-evolving functions for longer than two weeks even at room temperature indicating its very stable functional structure. Gel permeation chromatography shows purity and the size for the complexes, which is about twice as large as the 230-kDa fundamental PS II unit.

Association of PS II units to form dimers may occur when PS II units gain oxygen-evolving activity (Simpson and Andersson 1986). $CaCl_2$- and Tris-treatments dissociate the dimeric PS II complexes to the 230-kDa monomers, extrinsic proteins, and possibly Mn (Tanimura *et al*. 1994 , Takahashi *et al*. 1995). Single unit of PSII core complex has been proposed to associate making a homo-dimeric functional form from electron microscopic observation (Boekema *et al*. 1995) and radiation target analysis of the oxygen-evolving PSII core complex in thylakoid membranes (Takahashi *et al*. 1990). Thus, cooperation of two units of PS II core complex may be necessary for oxygen evolution function, however, direct evidence for the intrinsic structure of PS II has not been provided yet.

In this study we determined the *in vivo* structure of oxygen-evolving PS II core complex by comparing the subunit composition of PS II core complexes that were isolated from the mixture of thylakoids from *S. elongatus* wild type cells and *S. elongatus* cells expressing genetically-tagged subunit protein of PS II core complex.

Materials and methods

S. elongatus wild type cells, that were generous gifts from Dr. U. Mühlenhoff, Biologisches Institut II, Universität Freiburg, were transformed to express affinity-tagged CP43 (hereinafter, referred as to 43-H cell) as described by Sugiura and Inoue (1999). Thylakoid membranes were prepared from the 43-H cells and the wild type as described (Sugiura and Inoue 1999). Thylakoid membranes were solubilized by dodecyl maltoside (DM) and PS II core complexes that contained affinity-tagged CP43 were isolated from the DM-treated thylakoid membranes by Ni^{2+}-affinity chromatography according to Sugiura and Inoue (1999).

To elucidate the *in vivo* structure of PS II core complex, thylakoid membranes from *S. elongatus* 43-H cells were mixed with those from wild type cells with various ratio of from 1:0, 1:0.5, 1:1, 1:2 to 1:5 on the basis of the amount of chlorophyll prior to the disintegration of the thylakoid membranes with DM. Affinity-purified PS II core complexes were precipitated in 80% acetone solution at 4°C by centrifugation at 1,000 *g* for 10 min and suspended in 40 mM MES/NaOH (pH 6.5), 10 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, and 0.03% DM. They were solubilized with 1% SDS and then electrophoresed by an SDS-PAGE in a 16-22% gradient gel containing 7.5 M urea as described by Ikeuchi and Inoue (1988).

Results and discussion

The composition of subunit protein, especially of CP43, in affinity-purified PS II core complexes was inspected as a measure of artifactual dimerization of PS II core complexes in disintegrated mixture of supramolecular complexes of photosystems in thylakoid membranes. If the dimeric structure is the intrinsic functional PS II core complex, no dimeric PS II core complex could be composed of the subunit proteins from different source of thylakoid membranes.

An indigenous experiment was planned to answer whether PS II core complexes associate to form the functional dimeric structure intrinsically in thylakoid membrane or artifacturally after the DM treatment. In the latter case, monomeric PS II core complexes are active in the oxygen evolution function. Thylakoid membranes were prepared from *S. elongatus* cells that express CP43 subunit protein with COOH-terminus fused with His-tag and interconnecting thrombin cleavable site (CP43H). The thylakoid preparation was mixed with 0 to 5-times excess of the other thylakoid preparation of wild type *S. elongatus* and treated with DM. Ni^{2+}-affinity chromatography was used to isolate PS II core complexes with His-tagged CP43. If dimerization of PS II core complexes occur after the disintegration of thylakoid membranes by DM, PS II core complexes with a hybrid composition of His tagged CP43 and native CP43.

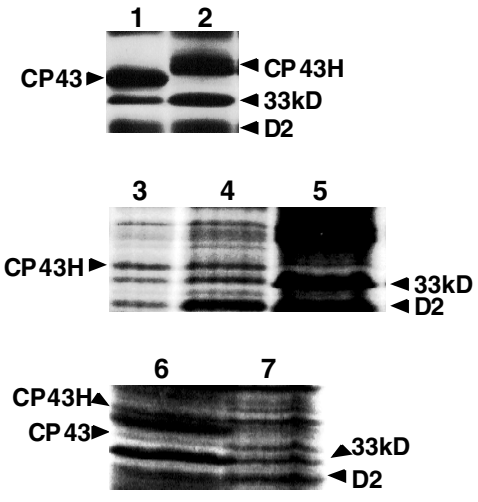

Figure 1: SDS-PAGE profiles showing the polypeptide compositions of the oxygen-evolving PS II core complexes purified from *S. elongatus* thylakoids by Ni^{2+}-affinity column. Lane 1: PS II cores from *S. vulcanus* wild showing the migration of CP43. Lanes 2 to 7: PSII core complexes purified from *S. elongatus* 43-H and wild type thylakoids mixed with the ratio of chlorophyll basis at 1:0, 1:0.5, 1:1, 1:2 and 1:5, respectively. Samples were loaded 5 μg each a lane.

Figure 1 shows CBB-staining patterns for D2, 33-kDa protein, and CP43 in SDS-PAGE of purified oxygen-evolving PS II core complexes. Lane 1 shows the migration pattern of PSII core complexes purified by Shen et al. (1992) from *S. vulcanus* wild type. CP43 of *S. vulcanus* wild type migrates faster than His-tagged CP43 of *S. elongatus* (CP43H; lane 2) although those of the other subunit proteins are same. *S. vulcanus* is also a thermophilic cyanobacterium with almost identical genome sequence for CP43 with *S. elongatus* (Hirano

M, personal communication). The COOH-terminal extension of CP43H might delay its migration in SDS-PAGE from CP43 of PS II core complex from untransformed *S. elongatus* cells. When the thylakoid membranes from *S. elongatus* 43-H cells were solubilized with DM together with the thylakoids from *S. elongatus* wild type, there were constant amount of CP43H in the affinity-purified PS II core complexes indicating quantitatively recovery of PS II core complexes having CP43H subunit. No CP43 was observed at all (lanes 3 - 6). In contrast, when large excess (5 times) of PS II core complexes of wild type was mixed, CP43-containing PS II core complexes associated with the PS II core complexes that contained CP43H subunit (lane 7).

Above results indicate that PSII core complexes occur as dimer before the detergent treatment, *i.e.* in thylakoid membranes. And the dimeric PS II core complexes seem to weakly interact each other to make oligomer in the detergent solution. This kind of association of PS II core complexes has been already recognized as a shoulder in the gel permeation pattern of PS II core complexes (Sugiura and Inoue 1999) and from the formation of a tetramer of spinach PS II core complexes during the long term centrifugation in the presence of octyl glucoside (Tanimura *et al*. 1994). PS II core complex loses its oxygen-evolving function in parallel with the loss of Mn and dissociation of intrinsic dimeric structure.

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