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Function of Extrinsic Proteins in Stabilization of the Photosynthetic Oxygen-Evolving Complex

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1. Introduction

Oxygenic phototrophs convert photon energy into chemical energy through a series of light-induced electron transfer reactions initiated with charge separation of chlorophyll (Chl) special pairs located in the central part of photosystem I and II (PSI and PSII) (Fig. 1). The reducing power is transferred from PSII to PSI through cytochrome b_6f, and finally utilized for reduction of $NADP^+$ to assimilate CO_2. The oxidized equivalents accumulated on the PSII donor side are neutralized by substrate water molecules to release protons for driving ATP synthase and O_2 molecules as a by-product. This water oxidation takes place in the oxygen-evolving complex (OEC) of PSII [McEvoy & Brudvig, 2006, Renger & Renger, 2008]. The OEC assembly is largely similar between cyanobacteria and higher plants, except for a critical difference in the composition of extrinsic proteins [Roose *et al.*, 2007]. In cyanobacteria, PsbO, PsbV, and PsbU residing on the lumenal side of PSII play significant roles in the regulation and stabilization of the water oxidation machinery. Higher plants possess major nuclear gene-encoded extrinsic proteins named PsbO, PsbP, and PsbQ. PsbO is a common extrinsic protein highly conserved among the oxygenic phototrophs. PsbP and PsbQ are indigenous to plant PSII and have been proposed as the functional equivalents of PsbV and PsbU in bacterial PSII, having replaced them during the course of evolution from ancestral cyanobacteria to higher plants. These proteins play significant roles in the regulation and stabilization of the photosynthetic water oxidation [Roose *et al.*, 2007, Seidler, 1996, Williamson, 2008] although the details of their function(s) are still a matter of debate.

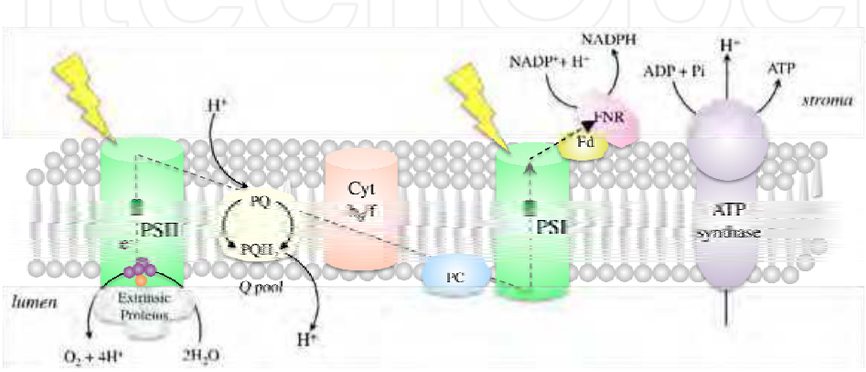


Fig. 1. Reaction scheme of photosynthesis in oxygenic phototrophs.

In this chapter, we describe the structural-functional roles of extrinsic proteins in the plant PSII. The effects of extrinsic proteins on the photosynthetic function of the Mn_4Ca cluster and the structural stability of the OEC core complex were investigated by spectroscopic and biochemical analyses. Based on the results presented here and reported previously, the structural and functional roles of extrinsic proteins in the regulation and stabilization of photosynthetic functions are discussed.

2. General structure and function of oxygenic phototrophs

2.1 Oxygen-evolving complex

Photosynthetic oxygen evolution occurs in the PSII OEC which is composed of a heterodimer of D1 (psbA) and D2 (psbD) proteins associated with two chlorophyll proteins (CP), CP47 (psbB) and CP43 (psbC), and involves a catalytic Mn_4Ca cluster located on the lumenal side of PSII (Fig. 2). These are highly conserved from cyanobacteria to higher plants to preserve the essential function of oxygenic phototrophs. The oxidized equivalents accumulated on the cluster and/or its ligands are reduced by electrons provided from a splitting reaction of substrate water molecules through a light-driven S-state cycle with five intermediate states S_n (n = 0 – 4), where n denotes the number of oxidizing equivalents stored. The OEC advances from the thermally stable S_1 state to the next oxidation state in a stepwise manner by absorbing each photon and attains the highest oxidation state S_4, followed by relaxation to the lowest oxidation state S_0 concurrent with a release of one oxygen molecule [Joliot *et al.*, 1969, Kok *et al.*, 1970]. Two water molecules are converted to one oxygen molecule by the OEC concurrent with release of four protons. Calcium and chloride ions are indispensable inorganic cofactors for playing functional and structural roles in the OEC [Debus, 1992, Yocum, 2008].

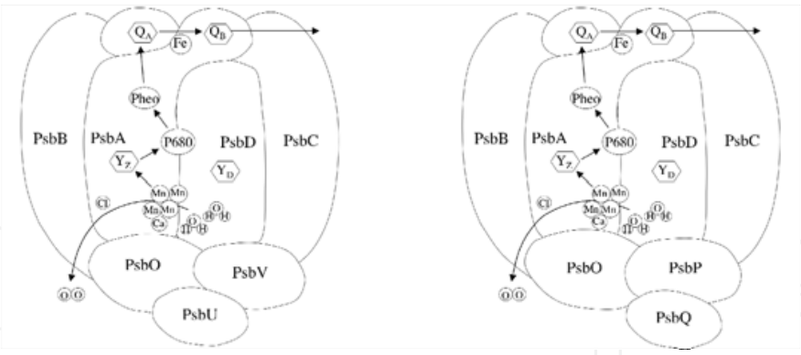


Fig. 2. OEC assemblies of cyanobacteria (left) and higher plants (right).

In the past decade, X-ray crystallography has revealed the structures of cyanobacterial PSII at resolutions of 3.8 Å to 2.9 Å [Ferreira *et al.*, 2004, Guskov *et al.*, 2009, Kamiya & Shen, 2003, Loll *et al.*, 2005, Zouni *et al.*, 2001]. A very recent structural model at the atomic resolution level has revealed details of the ligation structure of the Mn_4Ca cluster [Umena *et al.*, 2011]. The Mn ions are bridged by several oxygen atoms and coordinated by water molecules as well as by Asp, Glu, Ala, and His residues from PsbA and/or PsbC proteins. In contrast, high-resolution structural analysis of higher plants has been delayed due to the instability of the membrane protein complex. The visualization of plant PSII structures

has been limited to electron micrographs at low resolutions [Nield *et al.*, 2002, Nield & Barber, 2006]. Yet the findings to date strongly indicate that the structure and function of the PSII core assembly are almost identical to those of its prokaryotic counterparts, except for a critical difference in the composition of extrinsic proteins, which may provide valuable insights into the evolution of photosynthetic organisms [De Las Rivas *et al.*, 2004]. Based on the crystallographic structure of the OEC, several possible pathways for water, proton, and O_2 channels were proposed [Gabdulkhakov *et al.*, 2009, Guskov *et al.*, 2009]. However, photosynthetic water oxidation is a complex process that involves S-state cycling with five intermediate states, and therefore, the reaction mechanisms are not yet fully understood.

2.2 Extrinsic proteins in PSII

Higher plants possess gene-encoded extrinsic proteins, including PsbP, PsbQ, and PsbR, as well as PsbO, which commonly exists in all oxygenic phototrophs. These proteins play a key role in maintaining oxygen-evolving activity at physiological rates [Roose *et al.*, 2007, Williamson, 2008]. PsbO independently associates with the PSII core [Miyao & Murata, 1983, Miyao & Murata, 1989], and with PsbP through electrostatic interactions with PsbO [Miyao & Murata, 1983, Tohri *et al.*, 2004]. PsbQ requires both PsbO and PsbP for its binding [Miyao & Murata, 1983, Miyao & Murata, 1989]. In contrast, PsbO and PsbV independently bind to the PSII core, which lacks extrinsic proteins [Shen & Inoue, 1993], and the full binding of PsbU requires both PsbO and PsbV [Shen *et al.*, 1995, Shen & Inoue, 1993].

PsbP and PsbQ are thought to be the respective functional equivalents of PsbV and PsbU in the bacterial PSII [Enami *et al.*, 2005, Shen & Inoue, 1993], despite their low structural homology between PsbP(Q) and PsbV(U) [Balsera *et al.*, 2005, Ifuku *et al.*, 2004]. A phylogenetic study indicated that PsbP and PsbQ in the plant PSII were derived from PsbP and PsbQ homologues, respecively, in bacterial PSII [Thornton *et al.*, 2004], through intensive genetic modification during endosymbiosis and subsequent gene transfer to the host nucleus [De Las Rivas & Roman, 2005, Ifuku *et al.*, 2008, Ishihara *et al.*, 2007].

2.2.1 PsbO

PsbO is the most important protein for stabilization of the Mn_4Ca cluster, and therefore, it is common in all oxygenic phototrophs. The release of PsbO induces release of Mn ions from the cluster, resulting in the loss of O_2-evolving activity. The PsbO protein is common in every oxygenic phototroph but in varying proportions: one PsbO per PSII in cyanobacteria and two PsbO per PSII in higher plants [Williamson, 2008, Xu & Bricker, 1992]. High-resolution X-ray crystallographs of the PsbO protein associated with the PSII core are available for *Thermosynechococcus elongatus* [Ferreira *et al.*, 2004, Guskov *et al.*, 2009] and *Thermosynechococcus vulcanus* [Kawakami *et al.*, 2009, Umena *et al.*, 2011], in which PsbO is comprised of a β-barrel core with an extended α-helix domain. In contrast, the structural analysis of plant PsbO has been delayed and is limited to low-resolution images [Nield & Barber, 2006]. PsbO proteins are believed to play significant roles in protecting and stabilizing the catalytic center, however, none of the amino acid residue from PsbO serves as a direct ligand for the Mn_4Ca cluster.

Findings to date strongly indicate another significant role for PsbO: it is thought to modulate Ca^{2+} and Cl^- requirements for O_2 evolution [Seidler, 1996, Williamson, 2008]. However, the Ca^{2+}-binding properties of PsbO proteins are somewhat different between higher plants and cyanobacteria. It has been reported that plant PsbO induces structural changes upon the binding of Ca^{2+} [Heredia & De Las Rivas, 2003, Kruk *et al.*, 2003], which is not the functional Ca^{2+} necessary for the water oxidation [Seidler & Rutherford, 1996]. In contrast, no significant Ca^{2+}-induced stuctural change was found in cyanobacterial PsbO [Loll *et al.*, 2005], although it has been speculated that this protein serves as a low-affinity binding site for functional Ca^{2+} [Murray & Barber, 2006, Rutherford & Faller, 2001].

2.2.2 PsbP and PsbV

PsbP is also indispensable for the regulation and stabilization of PSII in higher plants [Ifuku *et al.*, 2008]. Deletion of this protein disables the normal functions of the plant PSII [Ifuku *et al.*, 2005]. This protein is related to the stability of the Mn_4Ca cluster as well as to the binding affinity of Ca^{2+} and Cl^- ions, which are essential cofactors for water oxidation reactions [Seidler, 1996]. Although it is unclear whether PsbP proteins directly interact with the Mn_4Ca cluster, the binding of this protein to the PSII core in the absence of Ca^{2+} is known to cause modification of its physicochemical properties, including redox potentials, magnetic structures, and ligation geometries of the Mn_4Ca cluster. It has also been speculated that PsbP is a metal-binding protein which reserves Mn^{2+} or Ca^{2+} to keep or deliver it to the apo-PSII [Bondarava *et al.*, 2005].

PsbV is also thought to be involved with the binding of inorganic factors. PsbV-lacking mutants were unable to grow photoautotrophically in the absence of Ca^{2+} or Cl^- [Shen *et al.*, 1998]. In contrast to PsbP, PsbV was capable of supporting water oxidation even in the absence of PsbO [Shen *et al.*, 1995], suggesting that it serves to maintain a proper ion environment within the OEC for optimal oxygen-evolving activity [Nishiyama *et al.*, 1994, Shen *et al.*, 1995, Shen & Inoue, 1993, Shen *et al.*, 1998, Shen *et al.*, 1995]. Although none of residue from PsbV serves as a direct ligand for the Mn_4Ca cluster, it has been suggested that this protein participates in stabilizing PsbA through electrostatic interactions [Sugiura *et al.*, 2010].

2.2.3 PsbQ and PsbU

The roles of PsbQ protein in photosynthetic functions are not yet fully understood. The PsbQ protein is not necessary for normal growth in higher plants [Ifuku *et al.*, 2005, Yi *et al.*, 2006]. However, this protein is required for photoautotrophic growth under low-light conditions [Yi *et al.*, 2006] and it is involved in the binding of functional Cl ions [Balsera *et al.*, 2005]. In cyanobacteria, PsbU-lacking mutants were capable of photoautotrophic growth in the absence of Ca^{2+} or Cl^-, but at reduced rates [Shen *et al.*, 1998]. The oxygen-evolving ability was reduced by the removal of PsbU and restored in part by the addition of Cl^- but not Ca^{2+}, indicating that PsbU regulates Cl^- requirement [Inoue-Kashino *et al.*, 2005, Shen *et al.*, 1997]. Another of its functions is the suppression of light-induced D1 degradation [Inoue-Kashino *et al.*, 2005]. Protection of the PSII core from reactive oxygen species (ROS) [Balint *et al.*, 2006] has also been proposed.

3. Interaction of extrinsic proteins with the Mn_4Ca cluster in the OEC

3.1 PsbP and PsbQ

3.1.1 Depletion/reconstitution of extrinsic proteins and functional Ca^{2+}

In this study, we used two types of PSII membranes lacking functional Ca^{2+} with or without PsbP and PsbQ. The sample preparation method is shown in Fig. 3. Berthold-Babcock-Yocum (BBY)-type PS II membranes (untreated PSII, A) were prepared from spinach according to the method described previously [Ono *et al.*, 2001]. The O_2-evolving activity was ~550 μmoles of O_2/mgChl/h. For depletion of Ca^{2+}, PsbP, and PsbQ, the membranes were suspended in medium A (2 M NaCl, 10 mM Mes/NaOH, and pH 6.5) at 0.5 mg of Chl per ml and gently stirred on ice under weak light（10 μmol/s/m^2）for 30 min. Next, the following procedures were carried out in complete darkness or dim green light unless otherwise noted: EDTA was added to the suspension to achieve a final concentration of 1 mM, followed by 10-min incubation in the dark. The suspension was centrifuged and extensively washed with Chelex-treated medium B (400 mM sucrose, 20 mM NaCl, 20 mM Mes/NaOH, and pH 6.5) to yield PSII membranes depleted of Ca^{2+}, PsbP, and PsbQ (ExCa^{2+}-depleted PSII, B). For depletion of PsbP and PsbQ proteins, PS II membranes were suspended in medium A at 0.5 mg of Chl per ml, and gently stirred on ice in darkness for 30 min. The extracted PsbP and PsbQ proteins were reconstituted into the NaCl/EDTA-treated PSII to obtain Ca^{2+}-depleted PSII (C).

Alternatively, the PSII membranes were washed with medium C (400 mM sucrose, 20 mM NaCl, 0.1 mM Mes-NaOH, and pH 6.5) and then treated with medium D (400 mM sucrose, 20 mM NaCl, 40 mM citrate-NaOH , pH 3.0) at 2 mg of Chl per ml. After 5 min incubation on ice in darkness, the suspension was diluted with medium D (400 mM sucrose, 20 mM NaCl, 500 mM Mops-NaOH, pH 7.5), and incubated for 10 min to facilitate the rebinding of extrinsic proteins. Then, the sample was washed with medium E (400 mM sucrose, 20 mM NaCl, 40 mM Mes/NaOH, 0.5 mM EDTA, pH6.5) to obtain PSII membranes depleted of only Ca^{2+} (lowpH-treated PSII ,D). Finally, the resulting low-pH-treated PSII membranes were treated with medium A to produce PSII membranes depleted of both Ca^{2+} and extrinsic proteins (ExCa^{2+}-depleted PSII, E).

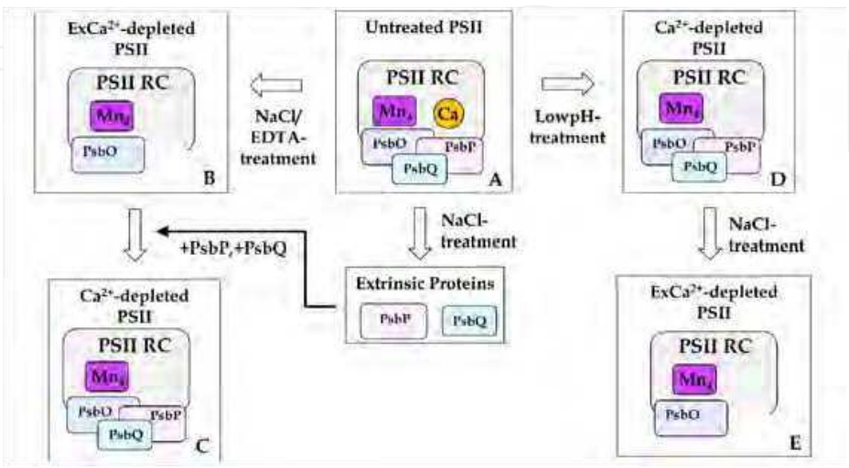


Fig. 3. Schematic representation for each sample preparation.

3.1.2 Effects of extrinsic proteins on the properties of the OEC

The PSII membranes prepared by the different methods were assessed by O_2-evolving activity and Fv/Fm values of chlorophyll fluorescence and the resulting data are summarized in Table 1. The O_2-evolving rate of the untreated PSII was decreased to 17% when PsbP, PsbQ and Ca^{2+} were depleted by the NaCl/EDTA treatments (B). The decreased activity was restored to 83% by reconstituting Ca^{2+}, as reported previously [Kimura & Ono, 2001, Ono *et al.*, 2001]. However, the addition of PsbP and PsbQ to the ExCa^{2+}-depleted PSII in the absence of Ca^{2+} lowered the O_2-evolving rate to ~0% (C). Furthermore, the O_2-evolving activity was almost completely lost upon Ca^{2+} depletion by the low-pH treatment (D) but restored to 79% by adding Ca^{2+}. Notably, the lost activity was partially resotored by the further depletion of PsbP and PsbQ to 25% (E). These results indicate that PsbP and PsbQ proteins completely suppress O_2 evolution in the absence of functional Ca^{2+}. Similar effects are also evident in the chlorophyll fluorescence measurements: the Fv/Fm values were much lower in the Ca^{2+}-depleted PSII (45%) than in the ExCa^{2+}-depleted PSII (64%), and both values were recoverd to ~80% after the supplementation with Ca^{2+}. In the Ca^{2+}-depleted PSII, the partial recovery to 68% was induced by the following depletion of the extrinsic proteins. Since Fv/Fm values are related to O_2-evolving activity, this strongly suggests that the functions of the OEC are disturbed by the extrinsic proteins in the absence of Ca^{2+}.

PSII preparation	Additives	O_2-evolving activity	Fv/Fm	FTIR S_2/S_1 carboxylate bands	Thermo-luminescence Q-band (˚C)	S_2 EPR multiline signal
Untreated PSII (A)	No addition	100%	100%	Normal[b]	Normal[e,f]	Normal[f]
ExCa^{2+}-depleted PSII (B)	No addition	17%	64%	Normal[b]	Normal[b,d,e]	Normal[d]
	+Ca^{2+}	83%	79%	Normal[b]	Normal[b,d,e]	Normal[d]
	+PsbP, +PsbQ (C)	~0%	---[a]	---[a]	Abnormal[e]	Modified
Ca^{2+}-depleted PSII (D)	No addition	~0%	45%	Abnormal[c]	Abnormal[e,f]	Modified[f]
	+Ca^{2+}	79%	81%	Normal[c]	Normal[e,f]	Normal[f]
	-PsbP, -PsbQ (E)	25%	68%	---[a]	Normal[e]	Normal

[a]No data, [b][Kimura & Ono, 2001], [c][Noguchi *et al.*, 1995], [d][Ono *et al.*, 2001], [e][Ono *et al.*, 1992], [f][Ono & Inoue, 1989].

Table 1. Effects of Ca^{2+} and extrinsic proteins (PsbP and PsbQ) on the properties of the OEC.

Next, the effects of extrinsic proteins and Ca^{2+} on the thermal stability of the OEC were examined. Fig. 4 shows the relative absorbance at 680 nm of the untreated control PSII (circle), ExCa^{2+}-depleted (triangle), and Ca^{2+}-depleted (square) PSII membranes during incubation at 50˚C. The relative band intensity of the control PSII remained at ~85% after 64 min incubation, but was slightly decreased to ~75% in the ExCa^{2+}-depleted PSII and was markedly decreased to 50% in the Ca^{2+}-depleted PSII. This is consistent with the effects seen in the O_2-evolving activity and Fv/Fm values. These results strongly support the idea that PsbP and PsbQ lower the structural stability and disturb the normal functioning of the OEC in the absence of Ca^{2+}.

The present findings are largely in agreement with previous findings, as shown in Table 1. FT-IR spectroscopy provides valuable information on the structure and interactions within the OEC. The ligation geometry around the Mn_4Ca cluster is mostly similar between untreated and $ExCa^{2+}$-depleted PSII, at least in the S_1- and S_2-states [Kimura & Ono, 2001]. However, Ca^{2+}-depleted PSII exhibited marked deterioration in the carboxylate bands, which are thought to be from putative amino acid residues coordinating to the Mn_4Ca cluster [Noguchi *et al.*, 1995]. Furthermore, the redox potential of the Mn_4Ca cluster has been reported to be abnormal when the extrinsic proteins bound to the PSII core in the absence of Ca^{2+}, as indicated by elevated peak temperatures of the thermoluminescence band for the

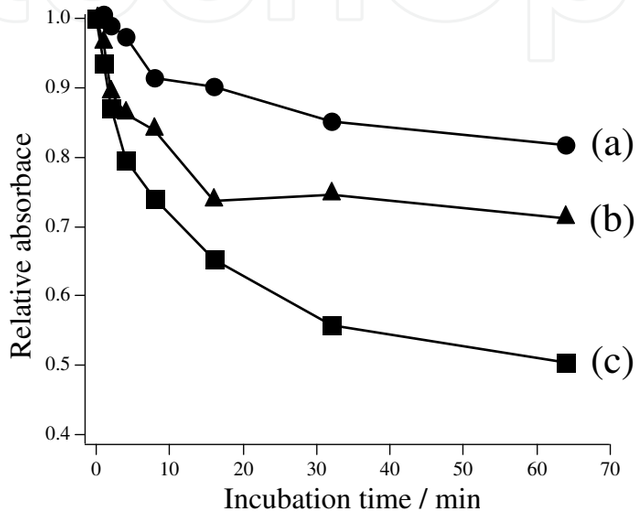


Fig. 4. Plots of relative absorbance at 680 nm as a function of incubation time at 50˚C for (a) untreated, (b) $ExCa^{2+}$-depleted, and (c) Ca^{2+}-depleted PS II membranes.

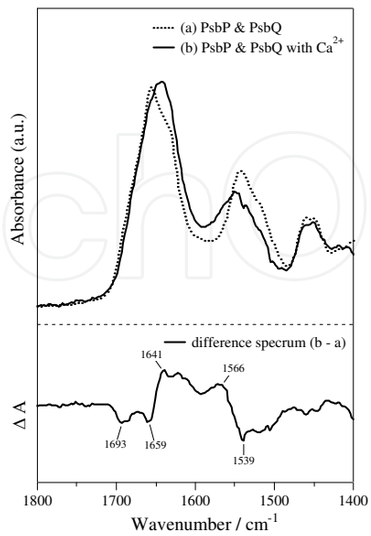


Fig. 5. ATR-FTIR spectra of isolated PsbP and PsbQ proteins in the absence (a, dotted line) and presence of Ca^{2+} (b, solid line). The difference spectrum obtained by subtracting spectrum a from spectrum b is shown in the lower panel.

$S_2Q_A^-$ recombination [Ono & Inoue, 1989, Ono *et al.*, 1992]. Additional support for this view was obtained from electron paramagnetic resonance (EPR) studies which demonstrated abnormal magnetic structures of PSII lacking Ca^{2+} but retaining the extrinsic proteins as revealed by modified S_2-state multiline signals. These results are largely compatible with the present findings that the appropriate binding of extrinsic proteins in the presence of functional Ca^{2+} is required for the normal functioning of the OEC.

To understand function of these extrinsic proteins in the OEC, structural changes of the PsbP and PsbQ proteins induced by Ca^{2+} were observed by ATR-FTIR spectroscopy. Fig. 5 shows ATR-FTIR spectra of isolated PsbP and PsbQ (spectrum a) and those supplemented with Ca^{2+} (spectrum b). The control spectrum a exhibited characteristic bands for amide I (1700 – 1600 cm^{-1}) and amide II (1600 – 1500 cm^{-1}) vibrational modes from backbone polypeptides of the OEC. These bands were significantly modified when Ca^{2+} was added to the extrinsic proteins as can be clearly seen in the difference spectrum (lower part of Fig. 5). The IR bands at 1693, 1659 and 1539 cm^{-1} are decreased and new bands are visible at 1641 and 1566 cm^{-1}, strongly indicating that PsbP and/or PsbQ are metal-binding proteins that alter their secondary structures upon the binding of Ca^{2+}. Similar structural changes were evident in the spectrum of the purified PsbP protein (data not shown). Although high-resolution crystallographic studies have revealed the structure of the PsbP protein in *Nicotiana tabacum* [Ifuku *et al.*, 2004], this protein lacks the N-terminal region which are thought to contain the Ca^{2+}-binding site, and therefore, the relationship between PsbP and Ca^{2+} remains unclear [Ifuku & Sato, 2002]. However, the authors of a previous study hypothesized that PsbP acts to reserve Mn^{2+} or Ca^{2+} ions [Bondarava *et al.*, 2005]. These results strongly support the idea that the PsbP protein is a metal-binding protein that directly and/or indirectly interacts with the catalytic center of the OEC in the absence of sufficient Ca^{2+}.

3.2 PsbO

The most important physiological role of PsbO is to stabilize the binding of the Mn_4Ca cluster, which is essential for oxygen-evolving activity [Debus, 2001]. The PsbO protein can be dissociated from the PSII by a variety of chemical treatments including washing with alkaline Tris buffer, a high concentration of $CaCl_2$, and chaotropic agents [Enami *et al.*, 1994, Ghanotakis & Yocum, 1990]. In particular, Lys residue-modifying chemicals such as *N*-succinimidyl propionate and 2,4,6-trinitrobenzene sulfonic acid caused release of PsbO from PSII and loss of oxygen-evolving activity [Miura *et al.*, 1997], suggesting that the positive charge of Lys is important for the electrostatic interaction between PsbO and PSII. Alternatively, the release of PsbO can be caused by thermal denaturation. However, PsbO itself is a thermostable protein [Lydakis-Simantiris *et al.*, 1999], and therefore, other factors might also be responsible for the release of PsbO as described later in this chapter.

Several spectroscopic studies using isolated PsbO reported different Ca^{2+}-binding properties between higher plants and cyanobacteria. It has been suggested that plant PsbO can bind Ca^{2+}, which induces slight changes in secondary structure from a β-sheet to a loop or nonordered structure, and facilitated the association of PsbO with the PSII core [Heredia & De Las Rivas, 2003, Kruk *et al.*, 2003]. However, an EPR study indicated that the functional Ca^{2+} ion was not involved in the binding to PsbO [Seidler & Rutherford, 1996]. In cyanobacteria, PsbO does not bind Ca^{2+}, at least before the protein associates with the PSII core, since no significant conformational change upon the Ca^{2+}-binding was induced in

isolated PsbO [Loll *et al.*, 2005]. In contrast, the low-affinity Ca^{2+}-binding site in PsbO located at the luminal exit of the proton channel has been suggested to be responsible for water oxidation [Murray & Barber, 2006, Rutherford & Faller, 2001]. These results strongly indicate that the structural-functional role of PsbO is not identical between higher plants and cyanobacteria. Interestingly, thermal stability was enhanced when plant PsbO proteins were replaced with thermally stable homologues from thermophilic *Phormidium laminosum* [Pueyo *et al.*, 2002]. Therefore, slight variation in the primary structure and/or the protein folding pattern is possibly responsible for the difference in thermal stability of PsbO between higher plant and thermophilic cyanobacteria.

4. Protective role of extrinsic proteins in regulation and stabilization of photosynthetic functions

4.1 PsbP and PsbQ

The present study revealed that PsbP significantly affects the structure and function of the Mn_4Ca cluster in the OEC only in the absence of sufficient Ca^{2+} in the OEC. This result is compatible with the previous analyses that involved FT-IR, thermoluminescence, and EPR spectoroscopies [Kimura & Ono, 2001, Noguchi *et al.*, 1995, Ono *et al.*, 2001, Ono & Inoue, 1989, Ono *et al.*, 1992]. In addition, it has been reported that PsbP has Ca^{2+}-binding sites in the N-terminal region [Ifuku & Sato, 2002] and functions as a reserver of Mn^{2+} or Ca^{2+} ions to supply them as needed by the impaired OEC [Bondarava *et al.*, 2005]. Therefore, it is possible that the PsbP completely eliminates functional Ca^{2+} or interacts with the Mn_4Ca cluster directly and/or indirectly to inhibit the O_2-evolving activity and modify the ligation geometry, redox potentials and magmetic structures of the Mn_4Ca cluster.

It has been suggested that normal functioning of PSII requires 15 highly conserved residues in the N-terminal region of the PsbP protein as well as the PsbQ protein for retention of functional Ca^{2+} [Ifuku *et al.*, 2005]. A recent FTIR study indicated that the PsbP protein, but not the PsbQ protein, has an effect on S_2/S_1 conformational changes of the intrisic polypeptide backbone around the Mn_4Ca cluster through the N-terminal region of the PsbP [Tomita *et al.*, 2009]. In addition, little change was found in characteristic carboxylate stretching modes from putative amino acid ligands for the Mn_4Ca cluster in the presence of Ca^{2+} when PsbP and PsbQ were depleted by NaCl washing, or all the extrinsic proteins were eliminated by $CaCl_2$ washing [Tomita *et al.*, 2009]. Based on these results, it is possible that the PsbP protein interacts with intrinsic proteins, which may be closely related to the Mn_4Ca cluster, and preserves the OEC functions appropriately in the presence of Ca^{2+}, but modifies the properties of the cluster directly and/or indirectly through intrinsic proteins in the absence of Ca^{2+}.

It is intriguing to note that PsbV in cyanobacteria exhibits functional similarity with PsbP in higher plants, although their primary and 3D crystallographic structures are largely different [Ifuku *et al.*, 2004, Kerfeld *et al.*, 2003]. The apparent inconsistency in the structural-functional consequence may reflect the fact that PsbP and PsbV in plant and cyanobacterial PSII are not involved in specific interactions between the protein and the Mn_4Ca cluster, but serve to maintain indispensable inorganic cofactors in the proximity of the cluster and to protect it from invasion. Additionally, PsbQ and PsbU also play a key role for tuning O_2-

evolving activity and enahncing structural stability through the interaction with PsbP and PsbV, respectively [Nishiyama *et al.*, 1997, Nishiyama *et al.*, 1999].

4.2 PsbO

In higher plants, PSII is much more susceptible to high temperatures than PSI [Berry & Bjorkman, 1980]. The thermal stability of the PSII core is closely related to the acquisition of cellular thermal tolerance in oxyphototrophs. The thermosensitivity of oxygen evolution in higher plants has been studied through simple experiments using PSII particles or isolated thylakoid membranes. Previous in-vivo and in-vitro studies have estimated the heat-labile properties of the OEC [Berry & Bjorkman, 1980, Havaux & Tardy, 1996, Mamedov *et al.*, 1993]. These studies demonstrated that the release of PsbO occurs first, followed by liberation of two of the four Mn ions from the Mn_4Ca cluster of the OEC [Enami *et al.*, 1998, Enami *et al.*, 1994, Nash *et al.*, 1985], and finally by the loss of oxygen evolution at high temperatures [Enami *et al.*, 1994, Yamane *et al.*, 1998].

Another form of damage to the physiological function of the PSII can be caused by reactive oxygen species (ROS) generated under high light conditions. The D1 proteins are degraded by the ROS species and inhibited in their ability to repair the photodamaged PSII by suppressing the synthesis of D1 proteins [Murata *et al.*, 2007]. The ROS species are thought to arise from heat-induced inactivation of a water-oxidizing manganese complex and through lipid peroxidation [Yamashita *et al.*, 2008]. On the other hand, saturation of polyunsaturated fatty acids (PUFAs) contributes to the acquisition of heat tolerance of photosynthesis by altering physicochemical properties [Alfonso *et al.*, 2001, Murakami *et al.*, 2000, Thomas *et al.*, 1986]. The increased saturation of PUFAs raises the temperature at which lipids phase-separate into non-bilayer structures, providing the proper assembly and dynamics of PSII tolerant to higher temperatures [Alfonso *et al.*, 2004].

Recently, we published biochemical evidence that the biological effect of reactive carbonyls such as malondialdehyde (MDA) and acrolein is greatly enhanced under heat-stressed conditions. [Yamauchi & Sugimoto, 2010]. PsbO is one of the proteins most frequently modified by MDA, which is an end-product of peroxidized polyunsaturated fatty acids. Detailed biochemical experiments indicated that the modification of PsbO by MDA affects its binding to the PSII complex and causes inactivation of the OEC (a schematic diagram is shown in Fig. 6). Purified PsbO and PSII membranes, from which extrinsic proteins had been eliminated, of the oxygen-evolving complex (PSIIΔOEE) of spinach were separately treated with MDA. The binding was diminished when both PsbO and PSIIΔOEE were modified, but when only PsbO or PSIIΔOEE was treated, the binding was not impaired. In an experiment using thylakoid membranes, the release of PsbO from PSII and a corresponding loss of oxygen-evolving activity were observed when thylakoid membrances were treated with MDA at 40°C but not at 25°C. In spinach leaves treated at 40°C under light, the maximum efficiency of PSII photochemistry (Fv/Fm ratio of chlorophyll fluorescence) and oxygen-evolving activity decreased. Simultaneously, the MDA content of the heat-stressed leaves increased, and PsbO and PSII core proteins (including 47 kDa and 43 kDa chlorophyll-binding proteins) were modified by MDA. In contrast, these changes were less profound when these experiments were performed at 40°C in the dark. Thus, MDA modification of PSII proteins likely causes the release of PsbO from PSII, an effect that is particuarly marked in heat and oxidative conditions.

First, ROS attack trienoic fatty acids in thylakoid membranes, resulting in the generation of MDA. MDA attaches to critical Lys residues of PsbO and PsbB (CP47) for the interaction between PsbO and PSII in a temperature-dependent manner. When both sides of PsbO and PSII are modified by MDA, PsbO is released from PSII. Finally, the Mn_4Ca cluster is spontaneously released from PSII, causing loss of oxygen-evolving activity.

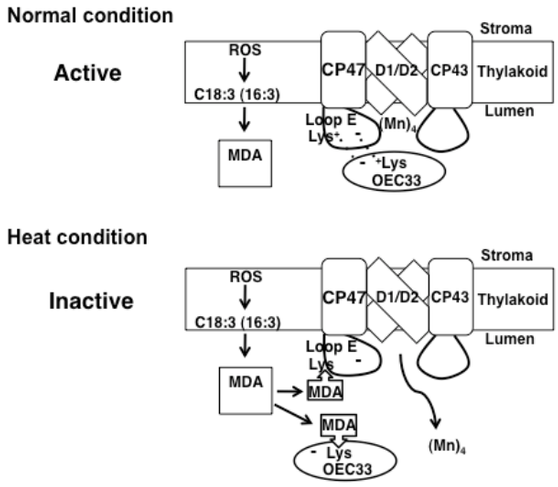


Fig. 6. A schematic model of MDA-induced loss of oxygen evolution in heat-stressed spinach PSII complexes.

5. Conclusion

In this article, we focused on the structural and functional roles of extrinsic proteins in the plant PSII. Since PSII is an integrated pigment-protein complex embedded in plant membranes, the structures and interactions of these extrinsic proteins in the membrane interface are of significance for protecting the RC. This involves protecting the Mn_4Ca cluster from exogenous invasion and/or alteration of physiological conditions. However, based on the results presented here and reported previously, we consider it very likely that the extrinsic protein itself is also responsible for the deterioration of the normal functioning of the OEC under inappropriate conditions. Further studies on the plant PSII, including high-resolution crystallographic strudies, will be required for understanding the functions of extrinsic proteins in the structural stability and the water oxidation chemistry in PSII.

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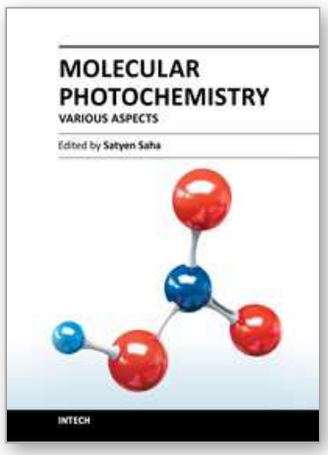
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There have been various comprehensive and stand-alone text books on the introduction to Molecular Photochemistry which provide crystal clear concepts on fundamental issues. This book entitled "Molecular Photochemistry - Various Aspects" presents various advanced topics that inherently utilizes those core concepts/techniques to various advanced fields of photochemistry and are generally not available. The purpose of publication of this book is actually an effort to bring many such important topics clubbed together. The goal of this book is to familiarize both research scholars and post graduate students with recent advancement in various fields related to Photochemistry. The book is broadly divided in five parts: the photochemistry I) in solution, II) of metal oxides, III) in biology, IV) the computational aspects and V) applications. Each part provides unique aspect of photochemistry. These exciting chapters clearly indicate that the future of photochemistry like in any other burgeoning field is more exciting than the past.

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