



Binding and functions of the two chloride ions in the oxygen-evolving center of photosystem II

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Abstract

Light-driven water oxidation in photosynthesis occurs at the oxygen-evolving center (OEC) of photosystem II (PSII). Chloride ions (Cl^-) are essential for oxygen evolution by PSII, and two Cl^- ions have been found to specifically bind near the Mn_4CaO_5 cluster in the OEC. The retention of these Cl^- ions within the OEC is critically supported by some of the membrane-extrinsic subunits of PSII. The functions of these two Cl^- ions and the mechanisms of their retention both remain to be fully elucidated. However, intensive studies performed recently have advanced our understanding of the functions of these Cl^- ions, and PSII structures from various species have been reported, aiding the interpretation of previous findings regarding Cl^- retention by extrinsic subunits. In this review, we summarize the findings to date on the roles of the two Cl^- ions bound within the OEC. Additionally, together with a short summary of the functions of PSII membrane-extrinsic subunits, we discuss the mechanisms of Cl^- retention by these extrinsic subunits.

Keywords Photosystem II · Oxygen-evolving center · Chloride ions · Extrinsic proteins · PsbP · PsbQ

Abbreviations

Cryo-EM	Cryo-electron microscopy
Cyt	Cytochrome
EPR	Electron paramagnetic resonance
FTIR	Fourier transform infrared
OEC	Oxygen-evolving center
PSII	Photosystem II
RNAi	RNA interference

Introduction

Photosystem II (PSII) is a multi-subunit pigment-protein complex found in the thylakoid membranes of oxygenic photosynthetic organisms (Debus 1992). Using light energy, PSII catalyzes the oxidation of water to molecular oxygen, producing most of the atmospheric oxygen on Earth (Dismukes et al. 2001; Barber 2016). The unique water-oxidizing reaction takes place in the oxygen-evolving center (OEC)

of PSII, which is formed by several membrane-intrinsic and membrane-extrinsic subunits (Shen et al. 2021).

The OEC contains an inorganic Mn_4CaO_5 cluster, consisting of four Mn ions, one Ca^{2+} ion, and five oxygen atoms arranged in a “distorted chair” form (Umena et al. 2011; Suga et al. 2015). In the water-oxidizing reaction, two water molecules are converted into one molecular oxygen, four protons, and four electrons in a stepwise manner through the S-state cycle (S_i ; $i=0-4$) of the OEC (Kok et al. 1970). S_1 is the most dark-stable state, and flash illumination advances each S_i ($i=0-3$) state to the next S_{i+1} state. Molecular oxygen is released in the final step from the transient, most oxidized S_4 state to the most reduced S_0 state. Several hydrogen-bonded water channels surround the Mn_4CaO_5 cluster, and may be involved in the inlet of substrate water molecules or the exit of produced protons (Murray and Barber 2007; Ho and Styring 2008; Gabdulkhakov et al. 2009; Umena et al. 2011; Vassiliev et al. 2012; Nagao et al. 2017c; Kern et al. 2018; Kaur et al. 2021). Additionally, two Cl^- ions specifically bind within the OEC, near the Mn_4CaO_5 cluster (Murray et al. 2008; Kawakami et al. 2009; Umena et al. 2011). They are related to some of the hydrogen-bond networks (Saito et al. 2013, 2015; Sakashita et al. 2017a).

Cl^- has long been known to be an essential cofactor for oxygen evolution by photosynthesis (Warburg and Lüttgens 1944; Arnon and Whatley 1949; Bové et al. 1963; for

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review, see Homann 2002), and was discovered to play a role in the water-oxidizing reaction of PSII (Heath and Hind 1969; Izawa et al. 1969). However, it had been challenging to determine the detailed functions of Cl^- and also the locations and number of binding sites (Debus 1992; van Gorkom and Yocum 2005; Yocum 2008). Some studies predicted one Cl^- binding site (Lindberg et al. 1993; Lindberg and Andrésson 1996), whereas other studies proposed two Cl^- binding sites (Boussac 1995; van Vliet and Rutherford 1996). Cl^- was implicated as a ligand to the Mn cluster in several studies (Critchley 1985; Rutherford 1989), while some studies using extended X-ray absorption fine structure (EXAFS) spectroscopy suggested the possibility that the Cl^- ions were not ligated to the Mn cluster (Yachandra et al. 1986a, b; Haumann et al. 2006). The binding sites of Cl^- were finally determined by X-ray crystallography; two Cl^- binding sites were found near the Mn_4CaO_5 cluster, and the Cl^- ions were not directly ligated to the Mn_4CaO_5 cluster (Murray et al. 2008; Kawakami et al. 2009; Umena et al. 2011) (Fig. 1).

Studies using green plant PSII showed that the membrane-extrinsic subunits are important for the retention of Cl^- ions required for optimal oxygen-evolving activity (Akabori et al. 1984; Andersson et al. 1984; Miyao and Murata 1985). As will be described in a later section (“Extrinsic subunits of PSII retaining Cl^- in the OEC”), differences are observed in the compositions of extrinsic subunits among the various oxygenic phototrophs (De Las Rivas et al. 2004; Bricker and Burnap 2005; Enami et al. 2005, 2008; Roose et al. 2007b, 2016; Bricker et al. 2012; Ifuku 2015; Ifuku and Noguchi 2016; Ifuku and Nagao 2021). However, extrinsic subunits in other species were also found to play a role in

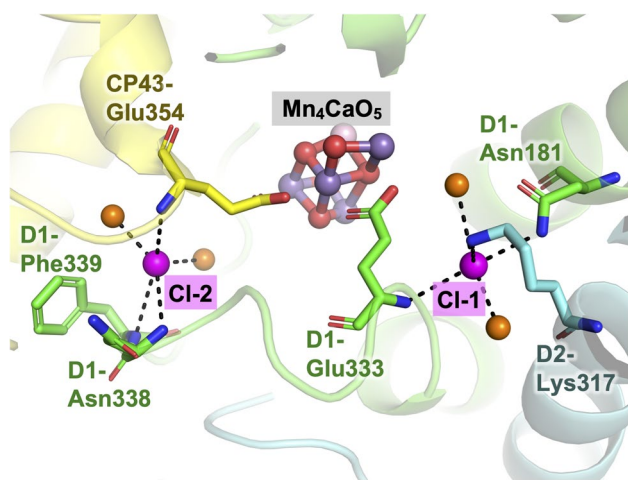


Fig. 1 Locations of the two Cl^- binding sites in the OEC of PSII (PDB ID: 3WU2). D1, D2, and CP43 are shown in green, blue, and yellow cartoon views, respectively. The Cl^- ions are shown as pink spheres, the water molecules nearby the Cl^- ions are shown as orange spheres, and the amino acid residues associated to the Cl^- ions are shown as stick models

Cl^- retention (Shen et al. 1997, 1998; Enami et al. 1998; Nagao et al. 2010a). The detailed mechanism of Cl^- retention by the extrinsic subunits remains to be fully elucidated.

Since the report of the cyanobacterial PSII crystal structure at 1.9 Å resolution (Umena et al. 2011), dramatic progress has been made in understanding the functions of Cl^- ions bound within the OEC. The functions of extrinsic subunits have been extensively studied as well. The recently reported PSII structures of various species (Ago et al. 2016; Wei et al. 2016; Su et al. 2017; Nagao et al. 2019; Pi et al. 2019) have facilitated a deeper understanding of their functions.

This review mainly focuses on the roles of the two OEC-bound Cl^- ions in the water-oxidizing reaction of PSII, as well as the structural mechanisms of Cl^- retention by membrane-extrinsic subunits. The roles of the Cl^- ions and the characteristics of extrinsic subunits are summarized, followed by a detailed discussion on the functional regions of extrinsic subunits that could be important for Cl^- retention in PSII. There have been excellent, comprehensive reviews that provide further information on the water-oxidizing reaction of PSII (McEvoy and Brudvig 2006; Vinyard et al. 2013; Yano and Yachandra 2014; Shen 2015; Lubitz et al. 2019).

Roles of Cl^- ions in the OEC of PSII

Effect of Cl^- ions on the water-oxidizing reaction by the Mn_4CaO_5 cluster

The two Cl^- binding sites in the OEC of PSII are called the Cl-1 and Cl-2 binding sites (Murray et al. 2008; Kawakami et al. 2009; Umena et al. 2011) (Fig. 1). The Cl-1 binding site, which had also been confirmed by Guskov et al. (2009), is surrounded by the amino group of D2-Lys317, the backbone nitrogen of D1-Glu333, and the side chain of D1-Asn181. The Cl-2 binding site is surrounded by the backbone nitrogen of D1-Asn338, D1-Phe339, and CP43-Glu354. The Cl^- ions are not directly ligated to the Mn_4CaO_5 cluster, but D1-Glu333 and CP43-Glu354 are both directly involved in the coordination of the Mn_4CaO_5 cluster, with D1-Glu333 serving as a bidentate ligand to Mn3 and Mn4, and CP43-Glu354 as a bidentate ligand to Mn2 and Mn3 (Umena et al. 2011).

Electron paramagnetic resonance (EPR) spectroscopy analyses, performed to study the structure and the magnetic properties of the Mn_4CaO_5 cluster (Debus 1992), demonstrated that Cl^- affects the Mn_4CaO_5 cluster in the S_2 state (Dismukes and Siderer 1981; Pokhrel et al. 2011). A characteristic $g = 2$ multiline signal was observed in the S_2 state in the presence of Cl^- (Dismukes and Siderer 1981). Depletion of Cl^- significantly lowered the above multiline signal

intensity, while giving rise to a different $g=4.1$ signal (van Vliet and Rutherford 1996). The EPR properties were recovered by resupplying Cl^- (Ono et al. 1986). Similar effects of Cl^- on the $g=2$ multiline EPR signal in the S_2 state were observed by comparing untreated PSII and extrinsic subunit-depleted PSII under different Cl^- conditions. The S_2 multiline signal was not significantly affected by the surrounding Cl^- concentration of untreated PSII (Franzén et al. 1985), whereas in NaCl-washed PSII, lacking extrinsic subunits (PsbP and PsbQ) required for Cl^- retention, the multiline signal was diminished by depletion of Cl^- from the buffer solution (Toyoshima et al. 1984; De Paula et al. 1986; Imakoka et al. 1986). Studies including Fourier transform infrared (FTIR) spectroscopy further showed that the Cl-1 site is structurally coupled with the Mn_4CaO_5 cluster (Service et al. 2013; Suzuki et al. 2013).

It has been suggested that the $g=2$ and $g=4.1$ forms indicate two different conformations (open-cubane and closed-cubane) of the Mn_4CaO_5 cluster in the S_2 state (Isobe et al. 2012; Pantazis et al. 2012; Bovi et al. 2013). It has been proposed that their stabilities are determined by the hydrogen-bond network around Cl-1, especially by proton transfer between D1-Asp61 and W1, a water molecule ligand to Mn4 of the Mn_4CaO_5 cluster (Pokhrel and Brudvig 2014; Amin et al. 2016; Yang et al. 2021). Recent theoretical studies have suggested that Cl-1 inhibits deprotonation of W2, another water molecule ligand to Mn4, and facilitates proton transfer from W1 via D1-Asp61 (Saito et al. 2020a; Mandal et al. 2022). Removal of Cl-1 has been shown to inhibit electron transfer from S_2 to Y_Z (D1-Tyr161) by increasing the redox potential (E_m) of S_2/S_3 (Mandal et al. 2022). The removal also leads to the formation of a salt bridge between D1-Asp61 and D2-Lys317, which would hinder the release of protons from D1-Asp61 via the water channel (Cl-1 channel) passing through this location (Rivalta et al. 2011; Amin et al. 2016; Mandal et al. 2022). Recently, it has been suggested that Cl-1 also affects the Mn_4CaO_5 cluster photoassembly in a similar way (Vinyard et al. 2019; Russell and Vinyard 2021).

The functions of Cl^- have also been studied using a combination of Cl^- substitution with other anions, and EPR spectroscopy or FTIR spectroscopy. Some of the most frequently used anions for Cl^- substitution are bromide (Br^-), iodide (I^-), fluoride (F^-), and nitrate (NO_3^-) ions. The oxygen-evolving activity of Cl^- -depleted PSII is greatly recovered by Br^- , partially by I^- and NO_3^- , and barely by F^- . The order of effectiveness in supporting the water-oxidizing reaction is $\text{Cl}^- \geq \text{Br}^- > \text{I}^- \geq \text{NO}_3^- > \text{F}^-$, with the effectiveness of I^- and NO_3^- inverted at higher concentrations (Hind et al. 1969; Kelley and Izawa 1978; Ono et al. 1987; Homann 1988a; Hasegawa et al. 2002). EPR studies have shown that while the S_2 multiline signal can be observed in Br^- -substituted PSII, it is remarkably diminished by

substitution with other anions, such as F^- (Casey and Sauer 1984; Damoder et al. 1986; Yachandra et al. 1986b; Ono et al. 1987). FTIR studies have investigated the effects of Cl^- on the conformational change of the OEC, mostly upon the S_1 -to- S_2 transition. The overall features of the FTIR spectra were similar among untreated PSII and PSII with Br^- , NO_3^- , or I^- substituted for Cl^- , although that of F^- -substituted PSII showed significant suppression of different bands (Hasegawa et al. 2002). However, the spectral features in the amide I region ($1700\text{--}1600\text{ cm}^{-1}$) that was changed by Cl^- depletion and recovered by Cl^- reconstitution were not restored by anion substitution with Br^- , NO_3^- , and I^- (Hasegawa et al. 2002; Kondo and Noguchi 2018). The effects on the amide I bands have been attributed to conformational changes, including those of the polypeptide chains surrounding the Cl^- binding site(s) (Kondo and Noguchi 2018). The X-ray crystallographic studies mentioned earlier confirmed that Br^- and I^- can bind to the Cl^- binding site (Murray et al. 2008; Kawakami et al. 2009; Umena et al. 2011). The collective findings suggest that, in addition to the negative charge, other properties such as the size of the Cl^- ion also affect the function of Cl^- in the water-oxidizing reaction.

Several studies have probed for the roles of Cl^- by introducing mutations at residues D2-Lys317 and D1-Asn181, both associated to Cl-1 with their side chains. In the D2-K317R mutant of *Synechocystis* sp. PCC 6803, Cl-1 remained bound, although with lowered affinity (Pokhrel et al. 2013; Suzuki et al. 2013). From the changes observed in the FTIR spectra (Pokhrel et al. 2013; Suzuki et al. 2013), EPR spectra, flash-induced O_2 yield patterns, and O_2 -release kinetics (Pokhrel et al. 2013) by this mutation, and $\text{Cl}^-/\text{NO}_3^-$ replacement in the wild-type (WT) strain and D2-K317R mutant (Suzuki et al. 2013), it was suggested that D2-Lys317 and Cl-1 have structural effects on the Mn_4CaO_5 cluster, and that they affect proton transfer through the Cl-1 channel. The D2-K317A, D2-K317Q, and D2-K317E mutants were also studied, and PSII with the D2-K317A mutation was found to lose Cl^- binding at the Cl-1 site while remaining active for oxygen evolution, although at a lower rate than WT PSII (Pokhrel et al. 2013). It was suggested that Cl-1 is required when the residue at the D2-Lys317 position is positively charged, in order to prevent the formation of a salt bridge with D1-Asp61. The lack of Cl^- requirement for oxygen-evolving activity in D2-K317A PSII may also suggest that binding of Cl-2 has little effect on the activity in D2-K317A PSII. Although D1-Asn181 is another residue associated to Cl-1, the D1-N181A and D1-N181S mutations showed some different effects compared to mutations at D2-Lys317 (Pokhrel et al. 2015). In both D1-N181A and D1-N181S PSII, Cl-1 remained bound, but the steady-state oxygen-evolving activities were lower than half of that of WT PSII. While the midfrequency

S₂-minus-S₁ FTIR difference spectra were similar to that with the D2-K317A mutation, flash-induced O₂ yield measurements revealed normal S-state cycling with a miss factor similar to that of WT PSII, although the O₂-release kinetics were slow. The authors concluded that the D1-N181A and D1-N181S mutations did not have significant effects on proton transfer, but slowed O–O bond formation by affecting the geometry of a substrate water. While a number of studies, including those mentioned above, have emphasized the importance of Cl-1 for the water-oxidizing reaction of PSII, to date, none have clarified whether or not Cl-2 is essential for the reaction.

Banerjee et al. (2018) showed that compared to cyanobacterial PSII, spinach PSII is more sensitive to Cl[−] depletion, and that the D1-N87A mutation in cyanobacterial PSII, which replicates residue 87 of D1 in spinach PSII, leads to similar Cl[−]-binding properties as spinach PSII. This D1-(Asn/Ala)87 residue had been found to be the most significant difference in D1 residues of cyanobacterial PSII and land plant PSII near the water channels (Vogt et al. 2015). In contrast to D1-N87A, the Cl[−] effect in D1-N87D mutant cyanobacterial PSII was similar to that of WT cyanobacterial PSII, while the S-state cycling was impaired unlike in D1-N87A (Banerjee et al. 2018). In the study, the authors concluded that the effect of Cl[−] (Cl-1) on the oxygen-evolving activity of PSII can be modulated by residue 87 of D1 by affecting the hydrogen-bond network that interconnects this residue with the Cl-1 site.

Effect of Cl[−] ions on water channels

As previously mentioned, the Mn₄CaO₅ cluster is surrounded by several networks of hydrogen-bonded water molecules (Umena et al. 2011). These water channels, some of which are affected by the Cl[−] ions as had been proposed by Olesen and Andréasson (2003), could be the pathways for the inlet of substrate water molecules or for the exit of protons. There are five main water channels: the O1, Y_Z-N298, Cl-1, O4, and Cl-2 channels (Nagao et al. 2017c; Sakashita et al. 2017a; Suga et al. 2019). The O1 channel, also referred to as the “large channel” (Ho and Styring 2008), proceeds from a large water cluster interacting with Y_Z (D1-Tyr161), D1-His190, and D1-Asn298, and passes through the O1 site of the Mn₄CaO₅ cluster and the D1-Glu329 residue (Murray and Barber 2007; Sakashita et al. 2017a, b; Suga et al. 2019). It should be noted, however, that while the O1 channel is observed in PSII structures from thermophilic cyanobacteria, this channel was recently reported to be blocked in PSII from *Synechocystis* sp. PCC 6803, a mesophilic cyanobacteria (Gisriel et al. 2022). The Y_Z-N298 channel starts from the same water cluster as the O1 channel but passes through D1-Asn332 (Umena et al. 2011; Nakamura et al. 2014; Shen 2015; Nagao et al. 2017c). The Cl-1 channel, also referred

to as the “E65/E312 channel” (Sakashita et al. 2017a) or the “broad channel” (Ho and Styring 2008), proceeds from D1-Asp61 through the Cl-1 binding site and the D1-Glu65/D2-Glu312 pair (Ferreira et al. 2004; Ishikita et al. 2006; Umena et al. 2011; Saito et al. 2013). The O4 channel, also referred to as the “narrow channel” (Ho and Styring 2008), starts from the O4 site of the Mn₄CaO₅ cluster and passes through D1-Asn338 (Saito et al. 2015; Takaoka et al. 2016; Sakashita et al. 2017a; Suga et al. 2019; Shimada et al. 2020; Kuroda et al. 2021). The Cl-2 channel consists of a hexagonal hydrogen-bonded network formed by Cl-2 and five water molecules near the Mn₄CaO₅ cluster (Sakashita et al. 2017a), and a water channel proceeding from the luminal bulk surface through PsbU-Asp96 and PsbU-Asn99, ending near the hexagonal hydrogen-bonded network (Umena et al. 2011). This channel is different from the aforementioned channels in that it does not connect the Mn₄CaO₅ cluster with the bulk surface because of interruption by the polypeptide backbone of the C-terminal region of D1 (Sakashita et al. 2017a). Despite extensive studies on these channels, no consensus has been established regarding the functions of each of them.

Of the five channels mentioned above, Cl-1 is involved in the Cl-1 channel (Fig. 2a). Although the Cl[−] binding sites had not yet been determined at that time, Ferreira et al. (2004) identified the channel corresponding to the Cl-1 channel in the PSII crystal structure that they obtained. The possibility that this channel functions as a proton-exit pathway or the water-inlet pathway was proposed (Barber et al. 2004; De Las Rivas and Barber 2004; Ferreira et al. 2004). A theoretical study by Ishikita et al. (2006) supported the view that this channel could function as a proton-exit pathway. Later, when Cl-1 was found to be located in this channel, it was proposed that Cl-1 could maintain the structure of this channel and/or proton transfer through this channel (Murray et al. 2008; Kawakami et al. 2009). Numerous theoretical and experimental studies have further investigated the function of the Cl-1 channel. Many studies have suggested that this channel is a proton-release pathway (Gabdulkhakov et al. 2009; Pokhrel et al. 2011; Rivalta et al. 2011; Ghosh et al. 2019; Ibrahim et al. 2020; Saito et al. 2020a; Hussein et al. 2021; Kaur et al. 2021; Kuroda et al. 2021). However, the channel may also play a role in the inlet of water molecules (Vassiliev et al. 2010, 2012; Sakashita et al. 2017a; Reiss et al. 2019; de Lichtenberg et al. 2021). As previously mentioned, Cl-1 has been suggested to be important for the structure of this channel by preventing the formation of a salt bridge between D1-Asp61 and D2-Lys317 (Rivalta et al. 2011; Pokhrel et al. 2013; Amin et al. 2016; Mandal et al. 2022), and for proton transfer through this channel (Kawashima et al. 2018; Saito et al. 2020a; Mandal et al. 2022).

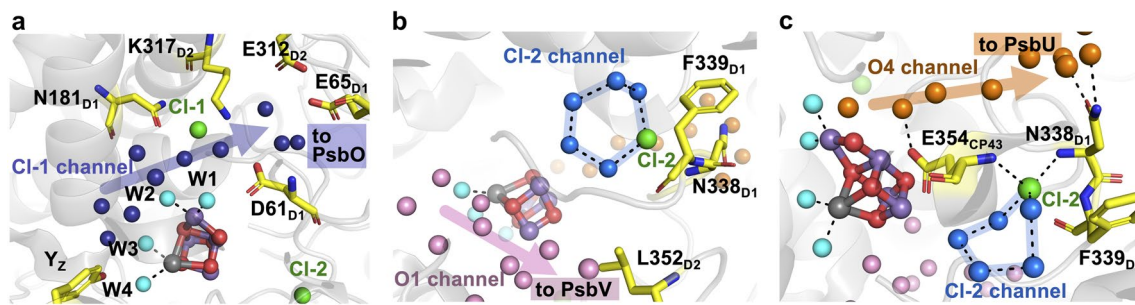


Fig. 2 Water channels found near the two Cl^- binding sites in the OEC of PSII (PDB ID: 3WU2). The two Cl^- ions are shown as green spheres, the four water molecules ligand to the Mn_4CaO_5 cluster (W1–W4) are shown as light blue spheres, and some of the residues nearby water channels are shown as yellow stick models. The arrows show the direction to the thylakoid lumen. **a** Cl-1 channel passing through the Cl-1 binding site. The water molecules participating in

the Cl-1 channel are shown as dark blue spheres. **b, c** Water channels related to Cl-2. **b** Cl-2 channel (hexagonal Cl-2–water network) consisting of Cl-2 and five water molecules, and O1 channel that could connect to the Cl-2 channel. Water molecules in the Cl-2 channel and the O1 channel are shown as blue and pink spheres, respectively. **c** O4 channel and Cl-2 channel. Water molecules in the O4 channel and the Cl-2 channel are shown as orange and blue spheres, respectively

In contrast to Cl-1, few studies have focused on the role of Cl-2 in hydrogen-bond networks. Of the five aforementioned channels, Cl-2 is related to the O4 channel and the Cl-2 channel (Fig. 2b, c). The O4 channel was first identified by Ho and Styring (2008) through solvent-accessibility simulations. The authors proposed that this channel could function as a proton-exit pathway. Many studies have suggested that the O4 channel plays a role in the release of protons (Gabdulkhakov et al. 2009; Saito et al. 2015, 2020b; Takaoka et al. 2016; Suga et al. 2017, 2019; Shimizu et al. 2018; Reiss et al. 2019; Ibrahim et al. 2020; Sakashita et al. 2020; Shimada et al. 2020; Yamamoto et al. 2020; Okamoto et al. 2021), whereas others have proposed its role in the inlet of water molecules (Vassiliev et al. 2010, 2012; Askerka et al. 2016; Retegan and Pantazis 2016; Retegan et al. 2016; Pantazis 2019). The most recent reports, including FTIR studies and theoretical studies taking the protein environment into account, support proton release through the O4 channel in the S_0 -to- S_1 transition (Saito et al. 2020a, b; Shimada et al. 2020; Yamamoto et al. 2020). Residues D1-Asn338 and CP43-Glu354, both coordinated with Cl-2, interact with water molecules involved in the O4 channel (Fig. 2c). CP43-Glu354 seems especially important for this channel, since it interacts with two of the four water molecules that are arranged in single file from O4 (Sakashita et al. 2020). Therefore, it can be assumed that Cl-2 is required to sustain the optimal structure of the O4 channel. It should be noted that while the O4 channel is suggested to be the proton-exit pathway upon the S_0 -to- S_1 transition (Saito et al. 2015), studies on the Cl^- requirement of the S-state cycle indicated that the S_0 state can advance to the S_1 state in the absence of Cl^- (Itoh et al. 1984; Wincencjusz et al. 1997). Cl-2 may be an efficiency-enhancing factor for proton release through the O4 channel.

In the Cl-2 channel, the other channel affected by Cl-2, Cl-2 participates in the hydrogen-bonded network itself (Fig. 2b, c). Nevertheless, in contrast to the intensively studied O4 channel, very few studies have focused on the Cl-2 channel. The Cl-2 channel was reported in the high-resolution crystal structure of PSII by Umena et al. (2011), and was initially proposed to function as a proton-exit channel or a water-inlet channel. However, the polypeptide backbone of the C-terminal region of D1 prevents the Cl-2 channel from connecting the Mn_4CaO_5 cluster to the bulk surface (Sakashita et al. 2017a). Until recently, no studies had explicitly investigated the function of the Cl-2 channel. A recent theoretical study based on hybrid ab initio quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations proposed proton release through this channel by a novel mechanism in which unidirectional proton transfer occurs through a peptide bond (Nakamura et al. 2019). However, this proposed mechanism has not been supported or refuted in other studies. Sakashita et al. (2017a) showed through MD simulations using the PSII structure of thermophilic cyanobacteria that the water molecules constructing the hexagonal hydrogen-bonded network together with Cl-2 originate from the O1 channel. The authors suggested that this hexagonal hydrogen-bonded network could be connected to the O1 channel by reorientation of the C-terminal D2-Leu352 residue. This could be the same in green plant PSII in which these channels have been found to be conserved (Sakashita et al. 2017b), although it may be different in mesophilic cyanobacterial PSII in which the O1 channel was observed to be blocked (Gisriel et al. 2022). Further investigation is required to determine the roles of the Cl-2 channel or the hexagonal Cl-2–water network.

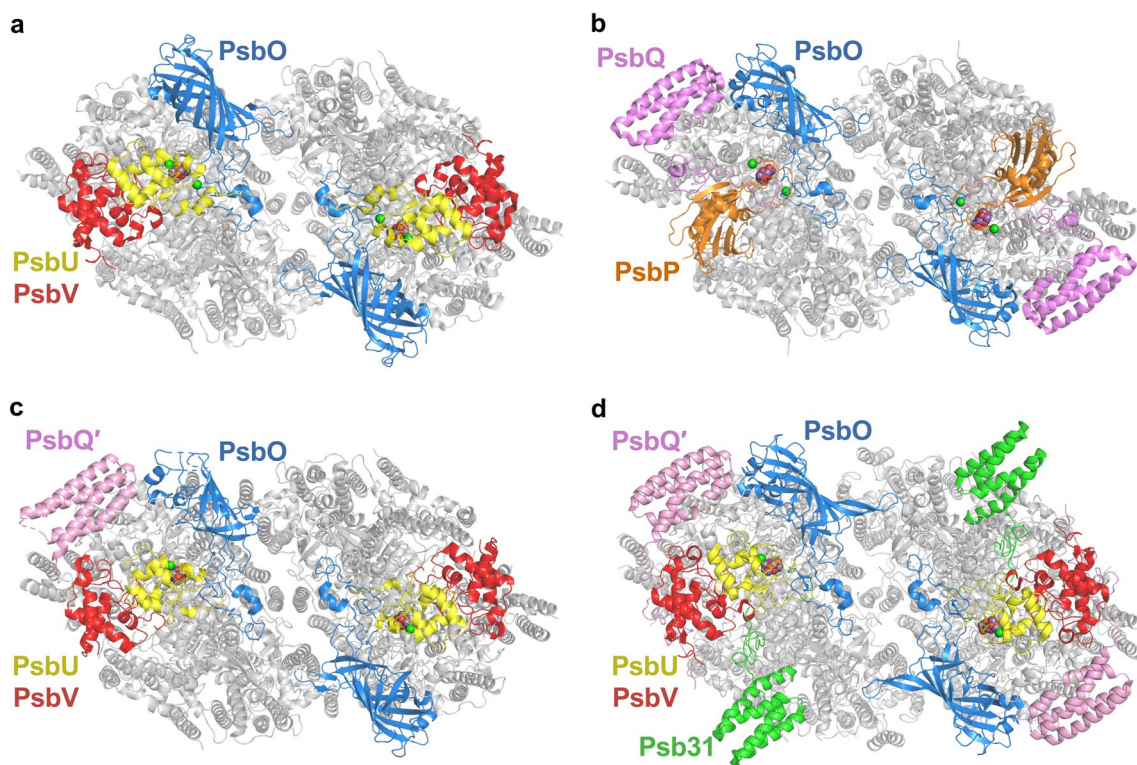


Fig. 3 Binding sites of extrinsic subunits in PSII from different species. PSII structures from **a** cyanobacteria (*Thermosynechococcus vulcanus*; PDB ID: 3WU2), **b** green plants (*Spinacia oleracea*; PDB ID: 3JCU), **c** red algae (*Cyanidium caldarium*; PDB ID: 4YUU), and **d** diatoms (*Chaetoceros gracilis*; PDB ID: 6JLU) are shown in cartoon view with the extrinsic subunits colored; PsbO (blue), PsbV

(red), PsbU (yellow), PsbP (orange), PsbQ and PsbQ' (pink), and Psb31 (green). The two Cl^- ions bound in the OEC are shown as green spheres, and the Mn_4CaO_5 cluster is shown with red, purple, and black spheres. Note that Cl^- is not observed in (c) and (d), presumably due to insufficient resolution or loss of the ion during experimental procedures

Extrinsic subunits of PSII retaining Cl^- in the OEC

Compositions and binding sites

The membrane-extrinsic subunits bind to the luminal side of PSII and surround the Mn_4CaO_5 cluster to form the OEC. Although the mechanism of water oxidation and the basic subunit structure of the PSII core are highly conserved among oxygenic photosynthetic organisms, ranging from cyanobacteria to diverse photosynthetic eukaryotes, drastic evolutionary changes have been observed in the composition of the extrinsic subunits (De Las Rivas et al. 2004; Bricker and Burnap 2005; Enami et al. 2005, 2008; Roose et al. 2007b, 2016; Bricker et al. 2012; Ifuku 2015; Ifuku and Noguchi 2016; Ifuku and Nagao 2021). Green plants, including land plants and green algae, have a set of three extrinsic subunits: PsbO, PsbP, and PsbQ (Åkerlund and Jansson 1981; Yamamoto et al. 1981; Kuwabara and Murata 1982). Only PsbO is always observed among all oxyphototrophs. In cyanobacterial PSII, PsbV and PsbU are present instead of PsbP and PsbQ (Bowes

et al. 1983; Stewart et al. 1985; Shen et al. 1992; Shen and Inoue 1993), although they possess CyanoP and CyanoQ, which are homologs of PsbP and PsbQ, respectively (Kashino et al. 2002; De Las Rivas et al. 2004; Thornton et al. 2004). Both CyanoP and CyanoQ are absent in the PSII structures of thermophilic cyanobacteria. However, a recently reported cryo-electron microscopy (cryo-EM) structure of PSII from a mesophilic cyanobacterium revealed the binding of CyanoQ in addition to PsbO, PsbV, and PsbU (Gisriel et al. 2022). Red algal PSII and diatom PSII bind PsbQ', a homolog of CyanoQ, in addition to PsbO, PsbV, and PsbU (Enami et al. 1995, 1998; Ohta et al. 2003; Nagao et al. 2007). Diatom PSII also binds an additional extrinsic subunit, Psb31 (Nagao et al. 2007, 2010b; Okumura et al. 2008).

X-ray crystallography and cryo-EM have revealed the binding sites of the membrane-extrinsic proteins in cyanobacterial (Umena et al. 2011; Suga et al. 2015; Gisriel et al. 2022), red algal (Ago et al. 2016), green plant (Wei et al. 2016; Su et al. 2017; Sheng et al. 2019), and diatom PSII (Nagao et al. 2019; Pi et al. 2019) (Fig. 3). PsbO binds to a similar location in different species. The

binding sites of PsbV and PsbU are identical among the PSII of cyanobacteria, red algae, and diatoms, and are spatially replaced by PsbP in green plant PSII. The PsbQ binding site of green plant PSII is either vacant or occupied by CyanoQ in cyanobacterial PSII, while it is occupied by PsbQ' in red algal and diatom PSII.

Fundamental functions

Although the composition of PSII extrinsic subunits varies among different species, the sets of these subunits seem to share fundamental functions. A major role of the extrinsic subunits is to form a functional, stable OEC that is shielded from the bulk solution. As can be seen in the PSII structures, the extrinsic subunits interact with luminal domains of the PSII core subunits, covering up the Mn_4CaO_5 cluster and Cl^- ions bound at the luminal surface of the membrane-intrinsic subunits (Umena et al. 2011; Ago et al. 2016; Wei et al. 2016; Nagao et al. 2019). Studies on green plant PSII have shown that depletion of extrinsic subunits leads to an exposed Mn_4CaO_5 cluster, enabling access of exogenous reductants (Ghanotakis et al. 1984a, c; Ifuku et al. 2005b). Similarly, it has been suggested that depletion of extrinsic subunits in cyanobacterial PSII destabilizes the OEC structure (Shen et al. 1995a, 1998; Inoue-Kashino et al. 2005). A recent study using high-speed atomic force microscopy (HS-AFM) on green plant PSII illustrated that extrinsic subunits are required to fix the flexible CP43 luminal domain (Pro304–Pro400), including residues directly interacting with the Mn_4CaO_5 cluster and Cl-2 (Tokano et al. 2020). Meanwhile, cryo-EM structures of assembly intermediates of cyanobacterial PSII, associated with assembly factors but not with the extrinsic subunits, suggested that even with the assembly factors bound, parts within the CP43 luminal domain become flexible (Huang et al. 2021; Zabret et al. 2021). Furthermore, FTIR studies in different species have shown that the extrinsic subunits are required for the proper conformational change of the OEC during the S_1 -to- S_2 transition (Tomita et al. 2009; Uno et al. 2013; Nagao et al. 2015). While shielding the OEC, the extrinsic subunits also participate in the construction of water-filled channels, allowing the inlet of substrate water molecules to the Mn_4CaO_5 cluster, as well as the release of protons from it (Bondar and Dau 2012; Vassiliev et al. 2012; Nagao et al. 2017c). A theoretical study showed that cyanobacterial PSII and green plant PSII have structurally conserved channels that pass through the different extrinsic subunits (Sakashita et al. 2017b).

Individual characteristics and functions

Numerous studies have focused on the structural and functional properties of individual extrinsic subunits. The data have been extensively summarized in previous reviews (Seidler 1996; Bricker and Burnap 2005; Roose et al. 2007b, 2016; Enami et al. 2008; Ifuku et al. 2008, 2011; Fagerlund and Eaton-Rye 2011; Bricker et al. 2012; Ifuku 2015; Ifuku and Noguchi 2016; Sasi et al. 2018; Ifuku and Nagao 2021). In this section, therefore, the characteristics and functions of each extrinsic subunit are described briefly.

PsbO, also designated OEC33, is the extrinsic subunit essential for the high oxygen-evolving activity of PSII in all oxygenic photosynthetic organisms. This subunit can solely bind to the PSII intrinsic proteins, and has a critical role in stabilizing the Mn_4CaO_5 cluster (Metz et al. 1980; Ghanotakis et al. 1984a; Miyao and Murata 1984; Kuwabara et al. 1985). FTIR studies have shown the requirement of PsbO for proper conformational change of the OEC upon the S_1 -to- S_2 transition (Nagao et al. 2015). PsbO also participates in the Cl-1 channel (Barber et al. 2004; De Las Rivas and Barber 2004; Ferreira et al. 2004; Ishikita et al. 2006; Ifuku and Nagao 2021), and partially in the O4 channel (Takaoka et al. 2016).

PsbV is a cytochrome (Cyt), designated as Cyt c_{550} (Shen et al. 1995b), but it has not been clarified whether it has redox functions in PSII (Guerrero et al. 2014; Khorobrykh et al. 2018). Cyanobacterial ΔPsbV mutant strains lacking PsbV were unable to grow photoautotrophically in the absence of Cl^- or Ca^{2+} (Shen et al. 1998; Katoh et al. 2001; Kirilovsky et al. 2004). A major function of PsbV is to critically support the retention of Cl^- and Ca^{2+} within the OEC, and this function is conserved among the PsbV proteins of cyanobacteria, red algae, and diatoms (Shen and Inoue 1993; Enami et al. 1998; Shen et al. 1998; Nagao et al. 2010a). Although cyanobacterial and red algal PsbV have differences in their binding properties (Enami et al. 2003) and their detailed effects on the conformational change of the OEC, FTIR studies have shown that they both have a role in arranging the structure of the OEC together with PsbO (Uno et al. 2013; Nagao et al. 2015). It has been suggested that PsbV is involved in the O1 channel and the Y_Z -N298 channel (Umena et al. 2011; Nagao et al. 2017c; Xiao et al. 2020; Ifuku and Nagao 2021).

PsbU is an extrinsic subunit with functions similar to those of PsbV; its binding is important for the retention of Cl^- and Ca^{2+} in cyanobacterial, red algal, and diatom PSII (Shen and Inoue 1993; Shen et al. 1997; Enami et al. 1998; Inoue-Kashino et al. 2005; Nagao et al. 2010a). Cyanobacterial ΔPsbU mutant strains lacking PsbU showed similar photoautotrophic growth rates as WT strains when grown in normal BG-11 growth medium. However, growth was reported to be partially impaired when Cl^- was depleted, and

even more so when both Cl^- and Ca^{2+} were depleted (Shen et al. 1997; Inoue-Kashino et al. 2005). Additionally, FTIR studies have shown that the binding of PsbU together with PsbO and PsbV contributes to the proper OEC structure in cyanobacterial and red algal PSII (Uno et al. 2013; Nagao et al. 2015). PsbU is also involved in the O4 channel (Saito et al. 2015; Takaoka et al. 2016; Ifuku and Nagao 2021).

PsbP, also designated OEC23 or OEC24, is an extrinsic subunit unique to green plant PSII (Åkerlund et al. 1982). In land plants, RNA interference (RNAi)-mediated knock-down of PsbP led to dramatically diminished photoautotrophy, emphasizing the importance of this subunit (Ifuku et al. 2005b; Yi et al. 2007). PsbP, which spatially replaces PsbV and PsbU in the other types of PSII (Wei et al. 2016; Ifuku and Nagao 2021) (Fig. 3), has functional roles similar to those of PsbV/PsbU. Binding of PsbP crucially supports Cl^- and Ca^{2+} retention (Andersson et al. 1984; Ghanotakis et al. 1984b; Miyao and Murata 1985, 1986; Homann 1987), and many different mutations in PsbP lead to a decreased Cl^- retaining ability (Miyao et al. 1988; Ifuku and Sato 2002; Ifuku et al. 2005a; Kakiuchi et al. 2012; Nishimura et al. 2014). FTIR studies have suggested that PsbP is essential for inducing proper conformational changes in the OEC (Tomita et al. 2009), and mutations in PsbP can perturb these conformational changes (Tomita et al. 2009; Ido et al. 2012; Kakiuchi et al. 2012; Nishimura et al. 2014). The FTIR study by Kondo and Noguchi (2018) further indicated that PsbP affects the conformational structures around the Cl^- ion(s). PsbP is also suggested to be involved in the O1 and O4 channels of green plant PSII (Sakashita et al. 2017b). In addition to these functions that resemble those of PsbV and PsbU, the N-terminal region of PsbP has been shown to interact with the α -subunit of Cyt b_{559} (PsbE) (Ido et al. 2012, 2014; Su et al. 2017) and modulate the redox potential of this cytochrome (Nishimura et al. 2016). Cyt b_{559} is indicated to be involved in an alternative electron flow (cyclic electron flow) within PSII, suppressing photoinhibition by accepting electrons from the reducing side of PSII and donating electrons to P680^+ (Radmer and Kok 1975; Shinopoulos and Brudvig 2012; Chu and Chiu 2016; Takagi et al. 2019). Therefore, PsbP is likely to have a role in fine-tuning the balance between the oxidation of water and the reduction of plastoquinone by PSII (Ifuku and Nagao 2021). The functional regions of PsbP will be further described and discussed in a later section (“Mechanisms of Cl^- retention by PsbP”) of this review.

PsbQ, also designated OEC16, OEC17, or OEC18, is the other extrinsic subunit unique to green plant PSII (Åkerlund et al. 1982). In contrast to PsbP-RNAi plants, PsbQ-RNAi plants exhibited no apparent phenotypes when grown under normal light conditions (Ifuku et al. 2005b; Yi et al. 2009). However, PsbQ-RNAi plants showed impaired autotrophy when grown under low light conditions (Yi et al. 2006).

In vitro, the binding of PsbQ together with PsbP supports the oxygen-evolving activity of PSII under very low Cl^- conditions (Akabori et al. 1984; Imaoka et al. 1984; Miyao and Murata 1985). FTIR analyses have shown that PsbQ has little influence on the conformational changes of OEC when WT-PsbP is bound to PSII, while it induced a proper OEC conformation that had been perturbed by mutations in PsbP (Kakiuchi et al. 2012). An auxiliary role of PsbQ in supporting PsbP binding has therefore been suggested, and the cryo-EM structure of green plant PSII supports this possibility (Wei et al. 2016). PsbQ also partially participates in the O1 channel of green plant PSII (Sakashita et al. 2017b). The domains of PsbQ will be further described in a later section (“Mechanisms of Cl^- retention by PsbQ”).

PsbQ', found in red algal and diatom PSII (Enami et al. 1995, 1998; Nagao et al. 2007), is an extrinsic subunit that has relatively low sequence similarity with PsbQ (e.g., 18.8% (40.9%) sequence identity (similarity) between PsbQ of *Spinacia oleracea* and PsbQ' of the red alga, *Cyanidium caldarium*) (Ohta et al. 2003; Nagao et al. 2013). Green algal PsbQ is more closely related to PsbQ' of red algae and diatoms than PsbQ of land plants; therefore, it can also be classified as PsbQ' (Yabuta et al. 2010; Ishikawa et al. 2020). While land plant PsbQ requires PsbO and PsbP for functional binding to PSII (Miyao and Murata 1989), PsbQ' can directly bind to PSII (Enami et al. 1998; Suzuki et al. 2003; Nagao et al. 2010a). FTIR analyses of red algal PSII suggested that binding of PsbQ' does not directly lead to a proper conformational change in the OEC upon the S_1 -to- S_2 transition (Uno et al. 2013). However, PsbQ' participates in supporting the effective binding of PsbV and PsbU to PSII (Enami et al. 1998; Nagao et al. 2010a).

Psb31 is the extrinsic subunit unique to diatom PSII (Nagao et al. 2007; Okumura et al. 2008). This subunit is structurally similar to PsbQ and PsbQ' (Nagao et al. 2013), but its binding site is different from those of PsbQ and PsbQ' (Nagao et al. 2017b; Pi et al. 2019) (Fig. 3). Reconstitution of Psb31 to PSII with no other extrinsic subunits, resulted in partial recovery of oxygen-evolving activity, suggesting that Psb31 has unique functions (Nagao et al. 2010a). It had been proposed that the flexible C-terminal region of Psb31 extends towards the OEC to affect the oxygen-evolving reaction (Nagao et al. 2017a, b), and a recently determined cryo-EM structure of diatom PSII confirmed that this C-terminal region reaches toward the OEC and contacts the Y_Z -N298 channel (Pi et al. 2019).

CyanoP and CyanoQ are lipoproteins found in cyanobacteria (Kashino et al. 2002; De Las Rivas et al. 2004; Thornton et al. 2004; De Las Rivas and Roman 2005). CyanoP has been suggested to be the ancestral homolog of PsbP (Ishihara et al. 2007), while CyanoQ has been suggested to be that of PsbQ and PsbQ' (De Las Rivas and Roman 2005). Until recently, neither CyanoP nor CyanoQ had been

observed in any of the crystal structures of cyanobacterial PSII. However, a recent cryo-EM structure of PSII from the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 revealed the binding of CyanoQ to PSII at a similar location as PsbQ or PsbQ' in PSII of other species (Gisriel et al. 2022). CyanoQ had been indicated to bind to PSII and optimize the oxygen-evolving activity, stabilizing the binding of PsbV (Kashino et al. 2006; Roose et al. 2007a). In contrast, CyanoP, which has not been found in cyanobacterial PSII structures, has been suggested to be an assembly factor of PSII (Cormann et al. 2014; Knoppová et al. 2016).

In terms of the detailed mechanisms of Cl^- retention by extrinsic subunits, this has been extensively studied through site-directed mutagenesis of PsbP of green plant PSII. However, most of these investigations were performed before the cryo-EM structures of green plant PSII were revealed. Thus, in the following sections, the functional regions of the Cl^- retention-related green plant PSII extrinsic subunits, PsbP and PsbQ, are discussed and compared with the extrinsic subunits in the other types of PSII, based on a combination of the results of previous studies and observations from the recently reported PSII structures.

Mechanisms of Cl^- retention by PsbP

As previously mentioned, in green plant PSII, PsbP is critical for Cl^- retention, contributing to the proper conformational structures around the Cl^- ion(s). However, which regions of PsbP are important for this function remains unclear. Release-reconstitution experiments with various mutated PsbP proteins have been performed to determine the functional regions for Cl^- retention by PsbP. Additionally, although the resolutions were not high enough to determine side-chain conformations, the recently revealed green plant PSII cryo-EM structures enable further discussions on the regions important for PsbP functions. This section discusses the roles of two candidate functional regions, the well-studied N-terminal region, and a most likely important loop named Loop 4, with a particular focus on the retention of Cl^- (Fig. 4).

N-terminal region of PsbP

The N-terminal region is the most intensively studied site within PsbP. This region consists of an N-terminal tail (Ala1–(Glu/Asp)17) and a subsequent β -hairpin structure located near the C-terminal helix (Fig. 4). Various deletions of nine to nineteen N-terminal residues of PsbP, decreased the oxygen-evolving activity of PSII and the binding affinity of PsbP to PSII (Miyao et al. 1988; Ifuku et al. 2005a, 2008; Kakiuchi et al. 2012). Even when amounts similar to WT-PsbP were bound to PSII, PsbP proteins depleted of fifteen

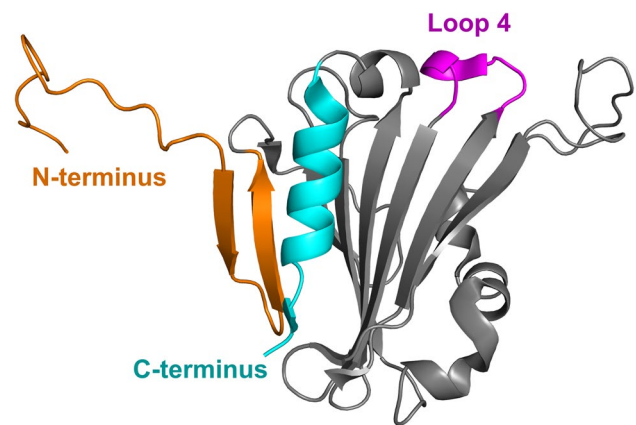


Fig. 4 Structure of PsbP (*Pisum sativum*; PDB ID: 5XNL). The N-terminal tail and β -hairpin are colored in orange, the Loop 4 region is colored in purple, and the C-terminal helix is colored in blue

and nineteen N-terminal residues ($\Delta 15$ - and $\Delta 19$ -PsbP) showed a remarkably decreased ability to retain Cl^- and Ca^{2+} . FTIR analysis of PSII reconstituted with $\Delta 15$ -PsbP revealed that the N-terminal residues were required for proper conformational changes around the Mn_4CaO_5 cluster (Tomita et al. 2009). On the other hand, mutation N15D decreased the oxygen-evolving activity, but did not have an apparent effect on the binding affinity of PsbP (Ifuku and Sato 2001). Furthermore, mutations K11A, K13A, and P20C had minor effects on the oxygen-evolving activity and PsbP binding affinity (Nishimura et al. 2014; Asada et al. 2018). Considering the cryo-EM structure (Su et al. 2017), residues in the Ala1–Phe9 region seem to be capable of interacting with PsbE, while residues in the Lys13–Thr16 and (Ala/Ser)32–Asn35 regions could contact a loop region of D1.

The above observations suggest that multiple interactions between the N-terminal region of PsbP and the membrane-intrinsic subunits are necessary for the functional binding of PsbP, while several particular residues could influence Cl^- retention and the oxygen-evolving activity of PSII. A D1-loop region found close to the N-terminal region of PsbP is located at the N-terminal side of a luminal helical structure of D1. Adjacent to the C-terminal side of this helix is the Cl-1 binding site (Fig. 5). Therefore, the interactions between the N-terminal region of PsbP and this loop of D1 could affect the binding of Cl-1. Kondo and Noguchi (2018) also pointed out that these interactions, as well as the possible interactions of PsbP-Asn52 and PsbP-Phe53 with the D1 luminal helical structure, could affect the Cl-1 binding site via this helical region.

The recently reported structures of diatom PSII show that the N-terminal region of PsbQ' binds along the interface of PsbV and membrane-intrinsic subunits, overlapping with the N-terminal region of PsbP in green plant PSII (Pi et al. 2019) (Fig. 6). It is possible that the N-terminal regions of

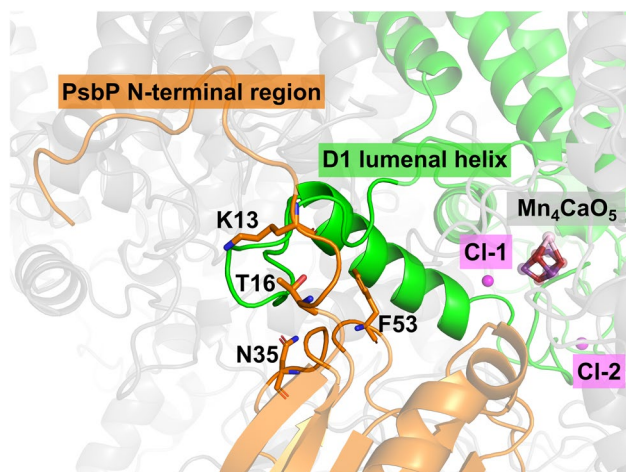


Fig. 5 Regions in PsbP nearby the luminal helical structure of D1 (PDB ID: 5XNL). Cl⁻ ions are shown as pink spheres, and D1 and PsbP are shown in green and orange cartoon views, respectively. Several PsbP residues that could interact with the D1 luminal helix are shown as stick models, and regions Lys13–Thr16, Ala32–Asn35, and Asn52–Phe53 of PsbP, and the region including the C-terminal luminal helix of D1 are highlighted

PsbP and PsbQ' have similar functions. This could partially explain why, unlike PsbQ (Miyao and Murata 1989), PsbQ' can solely bind to PSII (Enami et al. 1998; Suzuki et al. 2003; Nagao et al. 2010a), and why both PsbQ' and PsbO are required for full binding of PsbV (Enami et al. 1998; Nagao et al. 2010a), whereas PsbP only requires PsbO (Miyao and Murata 1989). In addition, although irrelevant to Cl⁻ retention, it is noteworthy that both PsbP and PsbQ' affect the redox properties of Q_A, even though these extrinsic subunits and Q_A bind to the other sides of the thylakoid membrane (Ono and Inoue 1986; Yi et al. 2007; Ido et al. 2009; Roose et al. 2010; Semin et al. 2018; Yamada et al. 2018; Kato and Noguchi 2021). This effect on Q_A could be related to the photoprotection of PSII (Kato and Noguchi 2022). The mechanisms of such transmembrane effects have

not been clarified, but could be related to the interactions of the N-terminal regions of PsbP and PsbQ' with membrane-intrinsic subunits, such as PsbE, and possibly PsbJ. In fact, PsbJ has been suggested to affect the redox potentials of Q_A and/or Q_B (Regel et al. 2001; Ohad et al. 2004; Boussac et al. 2021; Shen et al. 2021). Furthermore, as mentioned earlier, the interaction of the N-terminal region of PsbP with PsbE (the α -subunit of Cyt *b*₅₅₉) led to a transmembrane effect regulating the redox potential of Cyt *b*₅₅₉ (Nishimura et al. 2016). Further studies are required to confirm whether the N-terminal regions of PsbP and PsbQ' have common functions.

A study on the N-terminus-depleted PsbP proteins, Δ 15- and Δ 19-PsbP, also revealed the role of the N-terminal region in the tight folding of PsbP (Ifuku et al. 2005a). Compared to Δ 15-PsbP, Δ 19-PsbP proteins required lower temperatures for expression in *Escherichia coli* in a soluble form, and were easily digested by trypsin treatment. These findings suggest that residues 16 to 19 (Thr16–Met19 in spinach PsbP) may be important for the structural integrity of the PsbP protein. PSII cryo-EM structures (Wei et al. 2016; Su et al. 2017) and PsbP crystal structures (Ifuku et al. 2004; Kopecky Jr et al. 2012; Cao et al. 2015) show that of the above four residues, (Asp/Glu)17 and Phe18 could interact with the C-terminal helix (Gly171–Phe183) of PsbP. In the tobacco PsbP crystal structure (Ifuku et al. 2004), the carboxyl group of Glu17 is located 2.7 Å from the amino group of the conserved Lys174, and the side chain of Phe18 is located less than 4 Å from multiple residues (including the conserved Lys174 and Phe175) of the C-terminal helix (Fig. 7a). It is possible that these interactions are important for maintaining the overall structure of PsbP. In fact, the C-terminal helix of PsbP has been indicated to be critical for the overall structure of the protein. Depletion of the C-terminal helix of PsbP led to a drastic conformational change, hindering the import of PsbP to the thylakoid lumen, and stimulating its degradation (Roffey and Theg 1996). Interestingly, the crystal structure of DcrB, a PsbP-like-fold-containing

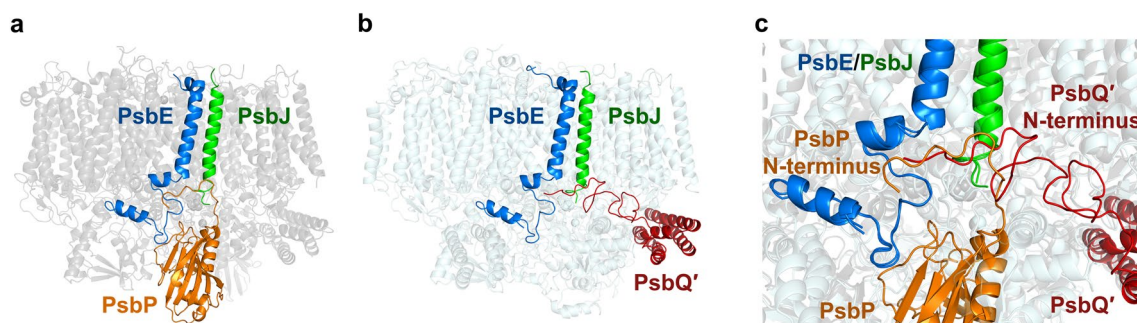


Fig. 6 Locations of the N-terminal region of PsbP in green plant PSII (PDB ID: 5XNL) and the N-terminal region of PsbQ' in diatom PSII (PDB ID: 6JLU). Location of **a** PsbP in green plant PSII and **b**

PsbQ' in diatom PSII. **c** Superposition of **a** and **b** with the N-terminal regions of PsbP/PsbQ' enlarged. PsbE, PsbJ, PsbP, and PsbQ' are shown in blue, green, orange, and red cartoon views, respectively

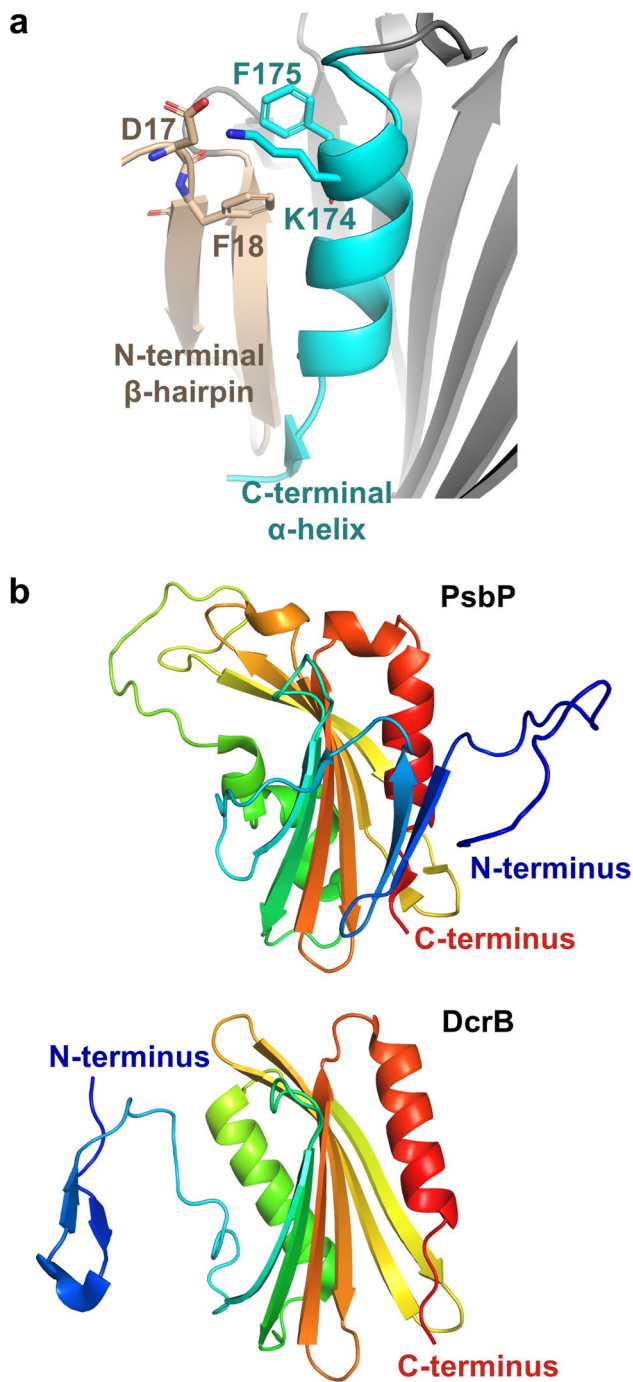


Fig. 7 **a** N-terminal β -hairpin structure (beige) and C-terminal α -helix (blue) of PsbP (PDB ID: 1V2B). Several residues that could have a role in the interaction between the N- and C-terminal regions are shown as stick models. **b** Comparison of the structure of PsbP (upper molecule; PDB ID: 5XNL) and DcrB (lower molecule; PDB ID: 6E8A). PsbP and DcrB are shown in rainbow-colored cartoon view with the N-terminus in blue and the C-terminus in red

lipoprotein found in *Enterobacteriaceae*, with a truncated N-terminal region revealed the formation of an N-terminal domain swapped crystallographic homodimer (Rasmussen

et al. 2018). The swapped domain was the N-terminal β -hairpin, which should interact with the C-terminal helix if it were within full-length PsbP (Fig. 7b). This indicates the potential flexibility of the N-terminal region of PsbP, and supports the possibility that perturbation of interactions between the N- and C-terminal regions destabilizes the conformation of PsbP. The cooperation of the N- and C-terminal regions of PsbP in supporting the oxygen-evolving activity of PSII (Ido et al. 2012) may also be related to this structural linkage between them.

Loop 4 region of PsbP

The Loop 4 region of PsbP (consisting of residues Thr135–Gly142) is found near the Cl-2 binding site, close to the C-terminal region of D2 and the C-terminal loop of D1. The distances between the polypeptide backbones (alpha carbons) of PsbP-Loop 4 and the C-terminal regions of D1 and D2 are, at the shortest, approximately 5 Å and 4 Å, respectively (i.e., 4.7 Å for PsbP-Glu140 – D1-Pro340, and 4.0 Å for PsbP-Asp139 – D2-Ala352). Although the side-chain conformations are not clear with the resolutions of the current green plant PSII cryo-EM structures, it is likely that these regions interact, or at least affect the conformations of each other, as has been proposed previously (Kondo and Noguchi 2018). Considering that Cl-2 interacts with the polypeptide backbones of D1-Asn338 and D1-Phe339, which are residues in the C-terminal region of D1, the PsbP-Loop 4 would almost directly affect the Cl-2 binding site. Therefore, PsbP-Loop 4 seems to be particularly important for Cl⁻ retention.

In cyanobacterial, red algal, and diatom PSII, the position of PsbP-Loop 4 is occupied by the C-terminus of PsbU (Fig. 8). Interestingly, previous studies using red algal PSII have indicated that this C-terminal region of PsbU is crucial in Cl⁻ retention (Okumura et al. 2001, 2007; Sano et al. 2008). These authors showed that the aromatic ring of the conserved tyrosine residue PsbU-Tyr92 (corresponding to PsbU-Tyr103 in cyanobacterial PSII) is important for optimizing Cl⁻ availability. The authors predicted that this attribute was related to the interaction of this conserved tyrosine residue with D1-Pro340, which was later revealed to be located near Cl-2. Taking this into consideration, PsbP-Loop 4 may have both spatially and functionally replaced the C-terminal region of PsbU.

Near PsbP-Loop 4 and the C-terminal region of D2, another conserved residue, CP47-Arg384, was found. The CP47-R384G mutant strain of cyanobacteria showed phenotypes, such as decreased oxygen-evolving activity, increased S₂ lifetime, and increased susceptibility to photoinhibition, although its growth rate was similar to that of the control strain (Putnam-Evans et al. 1996). The effects of this mutation and relevant mutations (e.g., R384G/R385G, R384E/

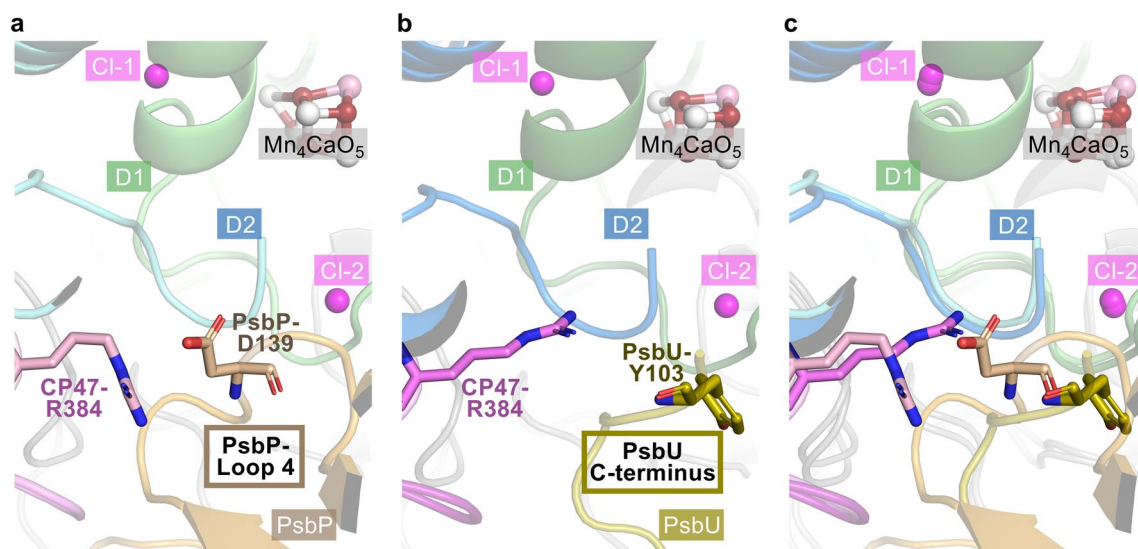


Fig. 8 Comparison of the structure around **a** PsbP-Loop 4 of green plant PSII (PDB ID: 5XNL) and **b** PsbU C-terminus of cyanobacterial PSII (PDB ID: 3WU2). **c** Superposition of **(a)** and **(b)**. D1, D2, CP47, PsbP, and PsbU are shown in green, blue, pink, beige, and

yellow cartoon views, respectively. The Cl^- ions are shown as pink spheres. CP47-Arg384, PsbU-Tyr103, and PsbP-Asp139 (found in green plant PSII at the location corresponding to the cyanobacterial PsbU-Tyr103) are shown as stick models

R385E, and Δ (R384-V392)) have been summarized (Bricker and Frankel 2002; Eaton-Rye and Putnam-Evans 2005; Morris et al. 2016). A recent study showed that the conformation of the loop region formed by CP47-Arg384 and CP47-Arg385 was changed by the binding of an assembly factor instead of extrinsic proteins (Huang et al. 2021). It is possible that the observed effects of the CP47-R384G mutation in cyanobacteria were, to some extent, due to its effect on the conformation of the C-terminal regions of PsbU and D2.

Mechanisms of Cl^- retention by PsbQ

In vitro, PsbQ supports the oxygen-evolving activity of green plant PSII under very low Cl^- conditions. However, in contrast to PsbP, PsbQ is located distant from the reaction center of PSII, and does not interact with regions near the Cl^- binding sites of PSII. Additionally, FTIR studies have shown that PsbQ has little influence on the OEC structure, at least during the S_1 -to- S_2 transition. Therefore, it remains unclear how PsbQ affects the Cl^- dependence of the oxygen-evolving activity.

Studies on the structure of PsbQ have shown that PsbQ can be divided into two regions: the core region folded as a four-helix bundle structure and the flexible N-terminal region (Balsera et al. 2003a, b, 2005; Calderone et al. 2003; Rathner et al. 2015) (Fig. 9). This is very similar to the structure of PsbQ' (Nagao et al. 2019). The possible roles of each region in supporting Cl^- retention are discussed below.

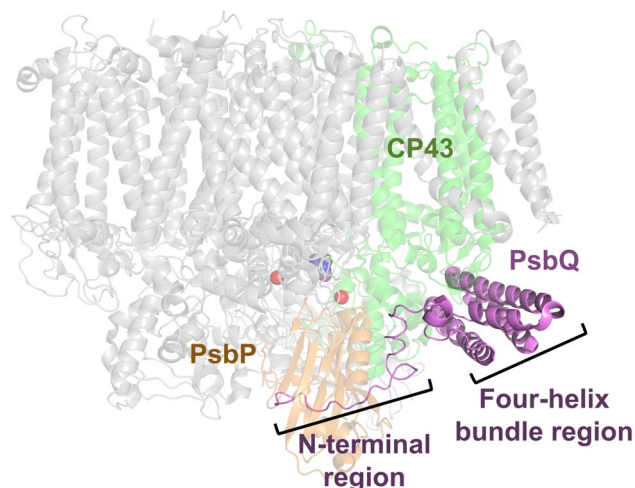


Fig. 9 Structure of PsbQ bound to green plant PSII (PDB ID: 3JCU). PsbQ, PsbP, and CP43 are shown in purple, orange, and green cartoon views, respectively

Four-helix bundle core of PsbQ

The four-helix bundle region of PsbQ binds to the luminal surface of CP43, placing the luminal domain of CP43 between PsbQ and the OEC (Wei et al. 2016; Su et al. 2017; Sheng et al. 2019) (Fig. 9). PsbQ', the PsbQ homolog found in red algae and diatoms, and CyanoQ, the PsbQ homolog found in cyanobacteria, have four-helix bundle cores similar to that of PsbQ, which bind to PSII at the same location as the PsbQ core (Ago et al. 2016; Nagao et al. 2019; Pi

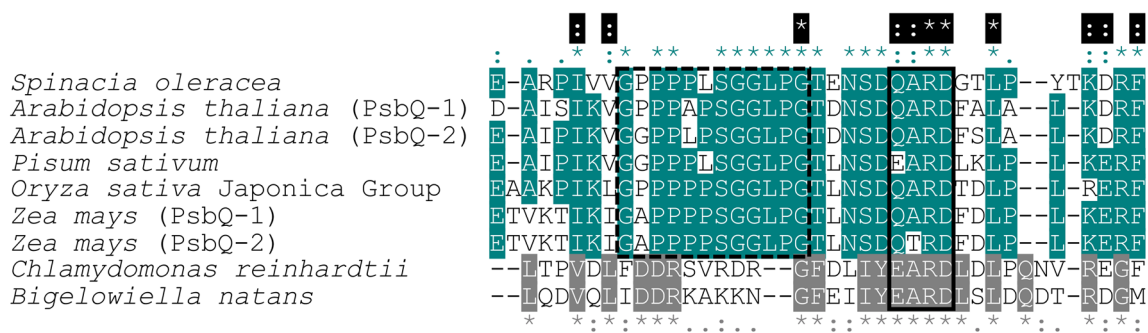


Fig. 10 Amino acid sequence alignment of PsbQ from land plants and green algae. Amino acids matching the consensus sequence are shown in blue (land plants) or gray (green algae) backgrounds. The asterisks (*), colons (:), and dots (.) indicate identical, conserved, and semi-conserved amino acids, respectively, and are colored blue for within only land plants, gray for within only green algae, and white

with a black background for both land plants and green algae. The box with black solid lines indicates (Gln/Glu)25–Asp28 (numbered based on spinach PsbQ), the region well conserved among PsbQ of land plants and green algae, and the box with black dotted lines indicates the proline- and glycine-rich regions of land plant PsbQ

et al. 2019; Gisriel et al. 2022) (Fig. 3). Interestingly, Psb27, an assembly factor involved in the biogenesis and repair of PSII (Mabbitt et al. 2014), also binds to this location with its four-helix bundle structure (Huang et al. 2021; Zabret et al. 2021; Gisriel and Brudvig 2022). Psb27 is associated with the luminal domain (Loop E) of CP43 (Liu et al. 2011, 2013; Komenda et al. 2012; Huang et al. 2021; Zabret et al. 2021), and it has been suggested that this interaction may stabilize the position of CP43-Loop E (Avramov et al. 2020). As previously mentioned, the luminal domain (Loop E) of CP43 is important for the OEC structure. Therefore, it is possible that the interaction of the PsbQ core or the PsbQ' core with CP43-Loop E has some influence on the structure of the OEC. However, FTIR studies have shown that, at least during the S_1 -to- S_2 transition, both PsbQ in green plant PSII and PsbQ' in red algal PSII have little effect on the structure of the OEC (Tomita et al. 2009; Uno et al. 2013). Additionally, depletion of PsbQ in green plant PSII does not affect the oxygen-evolving activity under Cl^- sufficient conditions (Miyao and Murata 1985; Homann 1988b). Moreover, binding of only PsbQ to green algal PSII, either with no extrinsic subunits or with only PsbO bound, does not recover the oxygen-evolving activity (Suzuki et al. 2003). Thus, the effect of the interaction between PsbQ and CP43-Loop E is more likely to be insignificant. Alternatively, the four-helix bundled core may be important for the binding of PsbQ to PSII (Meades et al. 2005), and bringing the N-terminal region to the right location.

N-terminal region of PsbQ

The N-terminal region of PsbQ binds along the luminal surface of PsbP. The N-terminal end of PsbQ is located near the PsbP–CP47 interface and extends towards the PsbP–CP43 interface (Wei et al. 2016; Sheng et al. 2019) (Fig. 9). In

land plant PsbQ, the N-terminal region contains a hydrophobic part rich in proline and glycine residues (Gly8–Gly19 in spinach PsbQ) (Balsera et al. 2003b) (Fig. 10). A prolyl endoproteinase specifically cleaves the N-terminal region of land plant PsbQ at the C-terminal side of Pro12 (Kuwabara et al. 1986; Kuwabara 1992; Kuwabara and Suzuki 1994). Release-reconstitution experiments were performed with the obtained PsbQ protein lacking the first twelve residues of the N-terminal region (Kuwabara et al. 1986). The N-terminal truncated PsbQ protein bound poorly to PSII and had lost its ability to support the oxygen-evolving activity at low Cl^- concentrations (Kuwabara et al. 1986). These findings illustrate the importance of the N-terminal region of PsbQ for the binding and function of this subunit. The first twelve residues of the N-terminal region of PsbQ include the proline-rich region (Pro9–Pro12 in spinach PsbQ). It has been suggested by a high-resolution PsbQ crystal structure that this region forms a polyproline type II (PPII) structure (Balsera et al. 2005), which could play a role in protein–protein interactions (Adzhubei et al. 2013; Narwani et al. 2017). However, this structure was not confirmed by nuclear magnetic resonance (NMR) spectroscopy (Rathner et al. 2015), and the green plant PSII cryo-EM structure does not have sufficient resolution needed for confirmation (Wei et al. 2016).

As described previously, the N-terminal region of PsbP is important for its function and structure, as well as for binding to PSII. $\Delta 15$ -PsbP, which is depleted of fifteen residues at the N-terminal end, does not functionally bind to PSII (Ifuku et al. 2005a; Tomita et al. 2009; Kakiuchi et al. 2012). However, PsbQ can restore the functional binding of $\Delta 15$ -PsbP to PSII (Kakiuchi et al. 2012). In the presence of PsbQ, $\Delta 15$ -PsbP showed a binding ability almost identical to that of WT-PsbP, and its ability to stimulate the oxygen-evolving activity was partially recovered. Similarly, while

the H144A mutation of PsbP resulted in reduced oxygen-evolving activity and Cl^- retention ability (Ido et al. 2012), these functional defects of H144A-PsbP were compensated for by the binding of PsbQ (Kakiuchi et al. 2012). FTIR analysis confirmed that the binding of PsbQ supports the correct conformational change of the OEC (Kakiuchi et al. 2012) which was perturbed in PSII reconstituted with only $\Delta 15$ -PsbP and H144A-PsbP (Tomita et al. 2009; Ido et al. 2012; Kakiuchi et al. 2012). These results indicate that PsbQ helps the binding of PsbP to PSII and supports the functional conformation of PsbP. The N-terminal region of PsbQ is closely associated with PsbP (Wei et al. 2016; Sheng et al. 2019). For example, the residues at the N-terminal end (Glu1–Pro9 in spinach PsbQ) are associated with a long flexible loop of PsbP (Wei et al. 2016), and some conserved residues such as (Gln/Glu)25 and Asp28 are associated with the C-terminal region of PsbP. Interactions between PsbP and the N-terminal region of PsbQ are likely to support the binding of mutated PsbP proteins to PSII. Moreover, interactions between the C-terminal helix of PsbP and the N-terminal region of PsbQ could be important in maintaining the proper linkage between the N- and C-terminal regions of PsbP, which was previously discussed as being critical for the overall conformation of PsbP.

The exact mechanism by which PsbQ supports the oxygen-evolving activity of WT PSII under low Cl^- conditions remains to be clarified. One conceivable hypothesis, based on the above discussion, is that the Cl^- retention ability of PsbP can be maximized by the N-terminal region of PsbQ supporting the binding and optimal conformation of PsbP. This effect of PsbQ could only be observed under low Cl^- conditions, possibly because the Cl^- retention ability of PsbP on its own is high enough for Cl^- retention under sufficient Cl^- conditions. Although green algal PsbQ does not contain the previously mentioned proline-rich hydrophobic region within its N-terminal region (Balsera et al. 2003b) (Fig. 10), it interacts with PsbP at locations very similar to that of land plant PsbQ (Sheng et al. 2019). Therefore, it is possible that the green algal PsbQ supports PsbP in a similar way as in land plant PSII.

In addition to the above hypothesis, another possible mechanism of the function of PsbQ can be proposed. In this mechanism, PsbQ regulates Cl^- accessibility through its effect on water channels within PSII. Sakashita et al. (2017b) showed that several water channels, including the O1 channel, are structurally conserved between thermophilic cyanobacterial PSII and green plant PSII. PsbQ-Asp28 (one of the few residues in the N-terminal region that is well conserved among land plant and green algal PsbQ (Balsera et al. 2003a, 2005) (Fig. 10)) and PsbP residues around the C-terminal helix of PsbP have been suggested to be located at the luminal end of the O1-PsbP

channel in green plant PSII (Sakashita et al. 2017b). In a different report, the water molecules in the Cl-2 cavity were suggested to originate from the O1 channel (Sakashita et al. 2017a). Therefore, the O1 channel is the water channel that may connect the Cl-2 binding site to the bulk solution, at least in thermophilic cyanobacterial PSII and green plant PSII. Thus, considering that PsbQ supports the retention of Cl^- under low Cl^- conditions, the N-terminal region of PsbQ may have some effect on the structure of the O1-PsbP channel, and could affect the diffusion of Cl^- ions through this channel. This proposed mechanism is not mutually exclusive with the first hypothesis, and may explain why the PsbQ N-terminal region (Glu/Gln)25–Asp28 is well conserved in both land plants and green algae, whose PSII bind PsbP (Fig. 10), but not in CyanoQ of cyanobacteria or PsbQ' of red algae and diatoms, whose PSII bind PsbV and PsbU instead of PsbP (Balsera et al. 2005; Gisriel and Brudvig 2022).

Conclusions

As summarized in this review, much progress has been made in understanding the roles of Cl^- ions within the OEC. Success in obtaining a high-resolution PSII crystal structure (Umena et al. 2011) has allowed detailed investigations on the roles of each of the two Cl^- ions bound near the Mn_4CaO_5 cluster. The importance of Cl-1 has been well studied, and it is becoming evident that its effect on the surrounding protein structure and hydrogen-bond network influences the water-oxidizing reaction. Additionally, the PSII structures from various species have provided valuable information, providing insights into the mechanisms of Cl^- retention by extrinsic subunits.

However, unanswered questions remain. As very few studies have focused on the functions of Cl-2, it is still unclear whether Cl-2 affects the oxygen-evolving activity of PSII, and if so, how. In terms of the mechanisms of Cl^- retention, some predictions can be made based on previous reports and PSII structures. However, further experimental evidence and PSII structures with higher resolutions (except for cyanobacterial PSII) would be required to determine these details.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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