9. Proton transfer in water oxidation

9.1. Proton exit pathway

9.1.1. Hydrophilic channels in the lumenal side of PSII

A number of mechanisms have been proposed for the redox reaction between P680 and the Mn-cluster. It is a matter of debate whether the role of Y_Z in water oxidation is to function as a hydrogen abstractor (Hoganson and Babcock, 1997; Vrettos and Brudvig, 2002) or electrostatic promoter (Ahlbrink et al., 1998). The existence of an exit pathway of protons released upon water oxidation without involving Y_Z and D1-His190 (proton exit pathway) was suggested by Haumann and Junge (Haumann and Junge, 1999). Connected to the Mn-cluster, a channel of polar residues starts at D1-Asp61 and precedes about 15 Å towards the docking site of PsbO on the lumenal surface as suggested by Barber, Iwata and coworkers (Barber et al., 2004; De Las Rivas and Barber, 2004; Ferreira et al., 2004; Iwata and Barber, 2004) (Figure 9-1-1). PsbO has a cylindrical β-barrel shape, but its interior is filled by hydrophobic residues including seven bulky Phe residues. Therefore, this protein is unlikely to function as a tube that channels water or protons (Barber et al., 2004; De Las Rivas and Barber, 2004). Instead, a cluster of hydrophilic residues at the entrance of the PsbO tube from the D1/D2 proteins seems to function as proton exit pathway with an overall length of about 35 Å consisting of D1-Asp61, D1-Glu65, D2-Glu312, D2-Lys317 (Ferreira et al., 2004; Iwata and Barber, 2004), PsbO-Asp158, PsbO-Asp222, PsbO-Asp223, PsbO-Asp224, PsbO-His228 and PsbO-Glu229 (Barber et al., 2004; De Las Rivas and Barber, 2004), without involving Y_Z and D1-His190. This channel may not only provide the proton exit pathway but also facilitate supply of water to the catalytic center (Barber et al., 2004). Due to the proximity of D1-Asp61 with one Mn ion of the Mn-cluster (Mn4 in ref. (Ferreira et al., 2004)), this residue was proposed to stabilize a water ligand to Mn (Barber et al., 2004). From a series of mutations, Chu *et al*. (Chu and Debus, 1995) suggested that D1-Asp61 and D1-Glu65 could influence the properties of the Mn-cluster without significantly affecting its structural features. Clausen *et al*. (Clausen et al., 2004) suggested the importance of D1-Asp61, because the mutation of D1-Asp61 to Asn delayed the half-rise time of O_2 release significantly. These results agree with the position of D1-Asp61 that was found in the neighborhood of the Mn-cluster (Ferreira et al., 2004). Based on the significant role of CP43-Arg357 in water oxidation (Knoepfle et al., 1999), McEvoy and Brudvig (McEvoy and Brudvig, 2004) further proposed that the proton moving along the exit pathway via D1-Asp61 might be abstracted from a water molecule by CP43-Arg357.

9.1.2. Calculated pK_a values for residues along proton exit pathway

From our computations we found a monotonous increase in pK_a of residues along the proton exit pathway from the Mn-cluster in D1/D2 to the lumenal surface in PsbO. According to these computations we suggest that in addition to the residues proposed by Barber, Iwata and coworkers (Barber et al., 2004; De Las Rivas and Barber, 2004; Ferreira et al., 2004; Iwata and Barber, 2004) also D1-Asp59, D1-Arg64 and PsbO-Arg152 may participate in the proton exit pathway. These three residues can form the chain of titratable residues with monotonous increase in pK_a, which are located along the proposed proton exit pathway (Figure 9-1-1) (Ishikita et al., 2005d). The energetically downhill slope in pK_a along the proton exit pathway is more pronounced in the S4 state than that in the S0 state. In particular the pK_a of D2-Lys317 at the entrance of the proton exit pathway is down-shifted drastically by 8 units (Figure 9-1-1)

upon transition of S0 to S4 state due to the increase of 4 unit charges on the Mn-cluster. For the same reason, the calculated pK_a values for D1-Asp61 or CP43-Arg357 are unusually low or high in the S4 state, respectively, while in the S0 state those pK_a are close to the standard values in aqueous solution. Production of an O_2 molecule from two water molecules at the Mn-cluster in PSII requires 3.2 eV, an enormous amount of energy (reviewed in ref. (Barber et al., 2004)). This energy is provided as oxidation power by the highly accumulated positive charge in the S4 state of the Mn-cluster, which is very unstable and has not been resolved in spectroscopic studies (reviewed in ref. (Goussias et al., 2002)). Therefore, it is not surprising that the residues in the neighborhood of the Mn-cluster possess unusually low or high pK_a values in the S4 state. Notably, such unusual pK_a shifts were computed only for residues in the immediate vicinity of the Mn-cluster (Figure 9-1-1) (Ishikita et al., 2005d). On the other hand, these residues that are likely to participate in the proton exit channel belong to a polar region in PSII such that the influence of the positive charge of the Mn-cluster will be shielded.

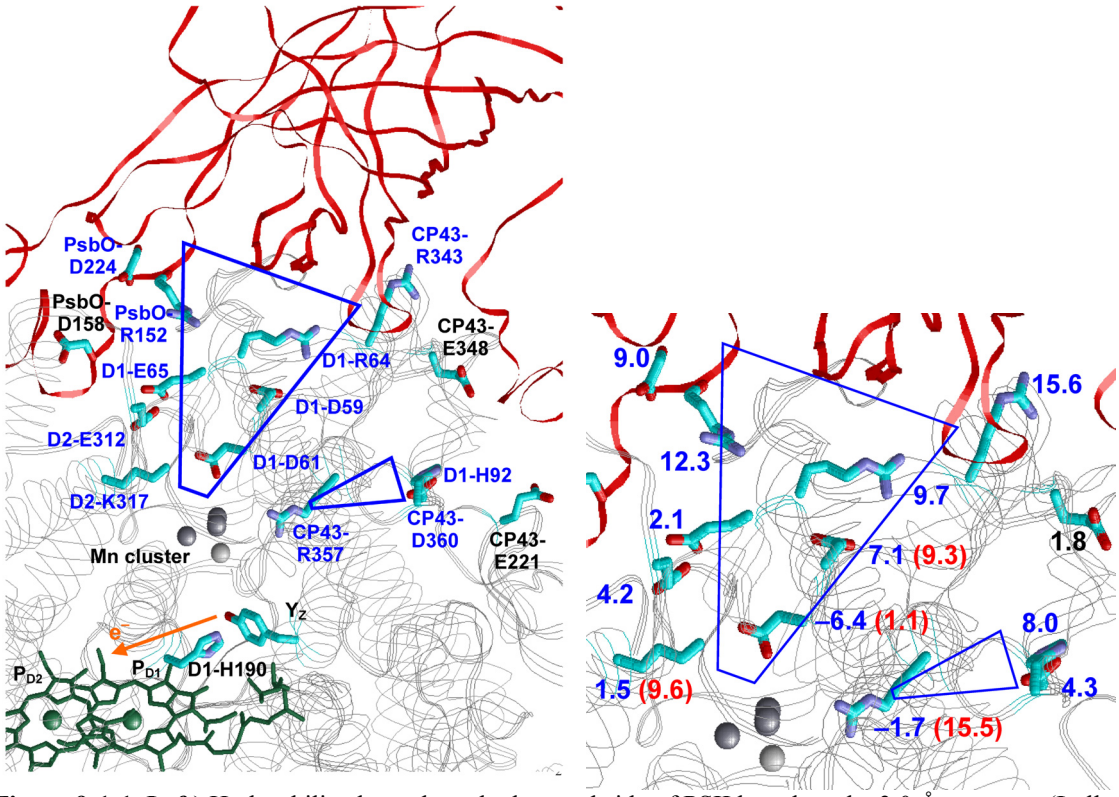

Figure 9-1-1. Left) Hydrophilic channels at the lumenal side of PSII based on the 3.0 Å-structure (Loll et al., 2005). Residues whose involvements in the proton exit pathway are highly probable based on the calculated pK_a in the present study are labeled in blue. PsbO is depicted as red ribbon. The blue lines mark the regimes of the proton exit pathway. For the sake of clarity, the other extrinsic proteins PsbU and PsbV are not shown in the figure. **Right)** Calculated pK_a values in the S4 state (colored in either blue or black) and in the S0 state (colored in red). The latter pK_a values are shown only for those residues that show significant differences (by more than 2) between the S0 and the S4 state.

9.1.3. D1-Asp59, D1-Arg64 and PsbO-Arg152 in the proton exit pathway

In addition to the residues proposed by Barber, Iwata and coworkers (Barber et al., 2004; De Las Rivas and Barber, 2004; Ferreira et al., 2004; Iwata and Barber, 2004), we suggest that D1-Asp59, D1-Arg64 and PsbO-Arg152 may also participate in the proton exit pathway (Ishikita et al., 2005d). Together with other titratable residues formerly

proposed, these three residues yield a down-hill energy proton transfer pathway starting from D1-Asp61 at the Mn-cluster and terminating at the lumenal surface of PSII independent of the redox state of the Mn-cluster (Figure 9-1-1).

There are experimental indications that D1-Asp59, D1-Arg64 and PsbO-Arg152 are associated with water oxidation in PSII. **(i) D1-Asp59:** Mutant studies of PSII from *Synechocystis* sp. PCC 6803 suggested that D1-Asp59 influences the properties of the Mn-cluster as well as D1-Asp61 and D1-Glu65, without significantly affecting the structural stability of the Mn-cluster. In these studies, the D(D1-59)N mutant showed lower light-saturated rates of oxygen evolution, implying that the rate of O_2 release was decreased (Chu and Debus, 1995). Furthermore, the reduced O_2 release in the D(D1-59)N mutant coincides with a decrease in overall PSII turnover during the S3-[S4]-S0 transitions, suggesting the importance of D1-Asp59 and D1-Asp61 to modulate the redox properties of the higher S-states (Qian et al., 1999). **(ii) D1-Arg64:** The R(D1-64)E mutant of PSII from *Synechocystis* sp. PCC 6803 showed a retarded appearance of O_2 and a pronounced tendency to lose O_2 evolution activity in the dark (Li and Burnap, 2001). **(iii) PsbO-Arg152:** Mutations of PsbO-Arg152 in PSII from *T. elongatus* resulted in a significant decrease of the binding affinity of PsbO to the PSII complex and decreased O_2 evolution ability, similarly as mutations of PsbO-Asp158 (Motoki et al., 2002). But, mutation of PsbO-Asp158 to Glu, a residue with the same unit charge as Asp, did not affect the PsbO binding affinity to PSII. In contrast, the mutation of PsbO-Arg152 to Lys resulted in a significant decrease PsbO binding affinity regardless of charge conservation at this residue, indicating the specific requirement of arginine at the position of PsbO-152 (Motoki et al., 2002). The retarded water oxidation and the associated processes for mutations at D1-Asp59, D1-Arg64 and PsbO-Arg152 may be associated with the interruption of the connectivity of the proton exit pathway and indicate the involvement of the three residues in the proton exit pathway.

9.1.4. Residues near the proton exit pathway

Based on the PSII crystal structure, CP43-Arg357 has been proposed to be a possible reaction site for the substrate water molecule in the neighborhood of Ca^{2+} (Barber et al., 2004; Ferreira et al., 2004; Iwata and Barber, 2004). Indeed, mutation of CP43-Arg357 to Ser resulted in a PSII with severely inhibited O_2-evolution (Knoepfle et al., 1999). It was further proposed that the proton moving along the exit pathway via D1-Asp61 might be abstracted from the water molecule by CP43-Arg357 (McEvoy and Brudvig, 2004). The change of protonation state for CP43-Arg357 upon S-state transition was proposed to play an important role in water oxidation (McEvoy and Brudvig, 2004). In our study, CP43-Arg357 is the residue that changes its pK_a most dramatically upon the S-state transition (Figure 9-1-1). In agreement with a previous proposal of McEvoy and Brudvig (McEvoy and Brudvig, 2004), this residue is fully deprotonated in the S0 state but fully deprotonated in the higher S state (the S4 state in the present study) (Figure 9-1-1) (Ishikita et al., 2005d).

In the PSII crystal structure (Loll et al., 2005), CP43-Asp360 forms an H bond with D1-His92 (O_{Asp}–$N\varepsilon_{His}$ distance of 3.1 Å) and both of the two residues are exposed to the lumenal bulk surface. D1-His92 is known to influence the catalytic efficiency of the Mn-cluster, without significantly affecting the structural stability of the Mn-cluster (Chu and Debus, 1995) in spite of the fact that D1-His92 is 17 Å from the Ca^{2+} in the current crystal structure (Loll et al., 2005).

9.1.5. An alternative channel and the Cd^{2+} binding site

Considering the above mentioned experimental results and the calculated pK_a values,

we propose that another chain of residues CP43-Arg357 – CP43-Asp360/D1-His92 may also function as either proton exit pathway for the Mn-cluster or as water intake channel from the bulk. Indeed, there is a relatively huge cavity between CP43-Arg357 and CP43-Asp360/D1-His92 at the binding interface between D1 and CP43 (Figure 9-1-1) (Ishikita et al., 2005d). Unlike the interior of the PsbO β-barrel, which is filled by a number of bulky hydrophobic residues, this channel is free of bulky hydrophobic residues.

The presence of D1-His92 at the entrance of this channel (Figure 9-1-2) is quite interesting, because His residues often appear at entry point of a proton transport channel. A metal ion binding at such a channel entry point His generally inhibits the physiological function of that channel. These His residues are found in the metal binding sites of bRC (Axelrod et al., 2000), cytochrome bc_1 complex (Berry et al., 2000) and cytochrome *c* oxidase (Aagaard and Brzezinski, 2001). Thus, in many different enzymes, transport channels employ His residues as entry points due to the proximity of its pK_a with the physiological pH of 7 (reviewed in ref. (Paddock et al., 2003b)). These structural analogies and similarities let us speculate that the pair of residues CP43-Asp360/D1-His92 form the entry point of the water transport channel at the lumenal side of the PSII complex (Figure 9-1-2). The titratable residue closest to CP43-Asp360/D1-His92 is CP43-Glu221. Hereby, participation of CP43-Glu221 in the entry point with the other two residues may be suitable to form the hexagonal Cd^{2+}-protein complex. However, an involvement of CP43-Glu221 may be uncertain, since CP43-Glu221 is 8.5 Å from D1-His92 (Figure 9-1-2a).

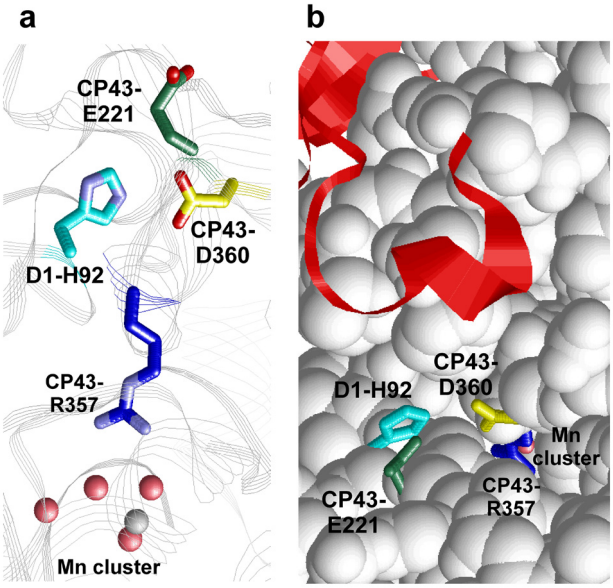

Figure 9-1-2. Water channel at the lumenal side. Only the side chains of D1-His92 (cyan), CP43-Glu221 (green), CP43-Arg357 (blue) and CP43-Asp360 (yellow) or the component of the Mn-cluster (Mn (pink) and Ca (gray)) are shown with stick or spacefill models. **a)** Side view. **b)** Top view: respectively. Proteins are uniformly depicted with spacefill model, except for PsbO with ribbon model.

It is known that Cd^{2+} binds to PSII at the donor side (O_2 evolving site) (Sigfridsson et al., 2004; Faller et al., 2005) and at the acceptor side ($Q_{A/B}$ site) (Sigfridsson et al., 2004). In connection with the proposed water channel at the donor side, two mechanisms could be considered. One possibility is that metal ions such as Cd^{2+} bind to PSII at the channel entry point as found in the other photosynthetic proteins. Based on Cd^{2+} affinity studies, Sigfridsson *et al.* (Sigfridsson et al., 2004) suggested that, among several possible Cd^{2+} binding sites in PSII, a low affinity site might involve a proton channel or H-bond chain from the catalytic site of water oxidation to the lumenal side of PSII, without excluding the other explanations. Hereby, it is possible that a modified proton release in water oxidation upon Cd^{2+} binding could change the turnover of the redox reactions involving Y_Z and the Mn-cluster (Sigfridsson et al., 2004). If so, then

the residues CP43-Asp360/D1-His92 may be the ligands for this Cd^{2+} binding site.

Another possibility is that Cd^{2+} binds competitively to the essential Ca^{2+} site at the Mn-cluster as suggested by Faller *et al*. (Faller et al., 2005) and Sigfridsson *et al* (Sigfridsson et al., 2004). The Mn-cluster is relatively easy to access from the lumenal surface through this alternative channel (Figure 9-1-2b). If the pair of residues CP43-Asp360/D1-His92 does not function as Cd^{2+} binding site, the prospective water channel may alternatively function as Ca^{2+} intake channel to bind Ca^{2+} at the Mn-cluster. It has been suggested that the lumenal extrinsic proteins play a role in optimizing the availability of Ca^{2+} and Cl^{-} for the Mn-cluster (reviewed in ref. (Seidler, 1996)). Indeed, this channel is located at the vicinity of PsbO (Figure 9-1-2). Therefore, it may also be possible that along this channel Ca^{2+} provided from PsbO competes with Cd^{2+} in binding at the Mn-cluster.

9.1.6. Influence of the extrinsic proteins on the energetics of the proton exit pathway

At the lumenal side, at least three proteins are attached to the D1/D2 complex of PSII. One of them, PsbO (33 kDa protein) is conserved in all PSII, showing a moderate primary sequence similarity of 40-50% between cyanobacteria and higher plants. The other two proteins are PsbU/PsbV in cyanobacteria and PsbQ/PsbP in green algae and plants. Regardless of similarity in association and function, these pairs of proteins differ considerably in their sequences and structures (see the structures of PsbU in refs. (Ferreira et al., 2004; Loll et al., 2005), PsbV in ref. (Frazao et al., 2001; Kerfeld et al., 2003), PsbP in ref. (Ifuku et al., 2004) and PsbQ in ref. (Calderone et al., 2003)). The extrinsic proteins PsbO, PsbU and PsbV have been suggested to optimize the availability of Ca^{2+} and Cl^{-} for the Mn-cluster. PsbO is also thought to contribute the structural stability of the Mn-cluster (reviewed in refs. (Seidler, 1996; De Las Rivas et al., 2004)). Thus, these extrinsic proteins at the lumenal side are closely related to the water oxidation reaction at the Mn-cluster. To investigate the influence of these extrinsic proteins on the energetics of the proton exit pathway, we calculated the pK_a shifts for those residues upon deletion of PsbO, PsbU or PsbV from the set of atomic coordinates of the PSII crystal structure (Loll et al., 2005).

The deletion of PsbO dramatically affected the pK_a of the residues, which are considered to participate in the proton exit pathway. D2-Glu312 and D1-Glu65 shifted their pK_a by ~1 unit, and D1-Asp59, D1-Arg64, CP43-Arg343 and CP43-Glu348 by more than ~2 units (Ishikita et al., 2005d). The significant decrease in pK_a of residues in CP43 upon deletion of PsbO may be related to the importance of CP43 in the assembly of the PSII complex, which was suggested to be a prerequisite for PsbO binding to PSII (Suorsa et al., 2004).

Upon deletion of PsbO, most of these residues except for D1-Asp59 are adapted to have more standard pK_a values in contrast to the unusual pK_a values in the presence of PsbO. Indeed, all these residues that change their pK_a upon deletion of PsbO are located on the binding interface of the PSII complex with PsbO, suggesting the strong interference of PsbO binding with the pK_a of those residues involved in the proton exit pathway. Regardless of the significant pK_a shift of the residues, the monotonous increase of pK_a along the proton exit pathway from the Mn-cluster to the lumenal surface is essentially maintained (Ishikita et al., 2005d). This may suggest that PsbO is not an absolute prerequisite for the energetics of the proton exit pathway but its presence is strongly required to guarantee normal proton exit events. In contrast to PsbO, deletions of PsbU or PsbV do not affect the pK_a of these residues (Ishikita et al., 2005d). The weak electrostatic influences of PsbU/PsbV on these residues are mainly due to the

large distances of more than 15/18 Å from the nearest residues of the proton exit channel, D1-Glu65/D1-Asp61, respectively.

The significant impact of PsbO binding on the proton exit pathway relative to the small influence on PsbU and PsbV is consistent with the conservation of PsbO in all species, which perform oxygenic photosynthesis. Indeed, among the lumenal extrinsic proteins of PSII, PsbO seems to be the most crucial to PSII. Enami *et al*. (Enami et al., 2000) revealed that PsbO isolated from cyanobacteria, red algae and higher plants is functionally interchangeable, indicating its universal role that is necessary for PSII function. In addition, PSII of the green oxyphotobacterium *Prochlorococcus marinus* possesses the *psbO* gene but lacks the genes encoding PsbU and PsbV. Based on this finding, De Las Rivas *et al*. (De Las Rivas et al., 2004) suggested that PsbO might be the minimal equipment with extrinsic proteins required for adequate functioning of water oxidation in PSII. The present study sheds light to the significance of PsbO in its functional role for the energetics of water oxidation in PSII i.e. tuning the pK_a of residues in the proton exit pathway along D1/D2/CP43/PsbO to foster the reaction efficiently.

Conclusion:

The pK_a computations in the present study suggest that **the proton exit pathway for water oxidation in PSII involves D1-Asp61, D1-Glu65, D2-Glu312, D2-Lys317 and PsbO-Asp224**. In addition, **D1-Asp59, D1-Arg64 and PsbO-Arg152 may also participate in the proton exit pathway. A second channel originates at CP43-Arg357 and terminates in a pair of residues CP43-Asp360/D1-His92** on the lumenal surface. This channel, which putatively functions as either proton exit or water intake channel, may be associated with the Cd^{2+} binding site of PSII. Among the lumenal extrinsic proteins, **PsbO has a significant impact on the pK_a of residues in the proton exit pathway**. Hence, a new role of PsbO is suggested: PsbO may also tune the pK_a of the residues in the proton exit pathway, thus contributing to the efficiency of water oxidation in PSII.