Oxygen-evolving complex of Photosystem II: An analysis of second-shell residues and hydrogen-bonding networks

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The oxygen-evolving complex (OEC) is a $\text{Mn}_4\text{O}_5\text{Ca}$ cluster embedded in the Photosystem II (PSII) protein complex. As the site of water oxidation, the OEC is connected to the lumen by channels that conduct water, oxygen, and/or protons during the catalytic cycle. The hydrogen-bond networks found in these channels also serve to stabilize the oxidized intermediates, known as the S states. We review recent developments in characterizing these networks via protein mutations, molecular inhibitors, and computational modeling. Based on these results, we highlight regions of the PSII protein in which changes have indirect effects on the S_1 , S_2 , and S_3 oxidation states of the OEC while still allowing photosynthetic activity.

Introduction

Photosystem II (PSII) is a light-driven water-plastoquinone (PQ) oxidoreductase. This large (700 kDa) homodimeric complex is embedded in the thylakoid membrane of cyanobacteria, algae, and higher plants. As revealed by structural studies in cyanobacteria [1], each monomer is composed of 17 transmembrane subunits and three extrinsic subunits; PsbO, PsbU, and PsbV (PsbO, PsbP, and PsbQ in higher plants) that bind to the luminal PSII surface. The PSII reaction center core consists of the subunits D1, D2, CP43, CP47, and cytochrome b_{559} (α and β subunits). The active site of water oxidation is the oxygen-evolving complex (OEC) where O_2 is produced and protons are released into the lumen (eq 1). The OEC consists of a Mn_4CaO_5 inorganic cluster ligated by waters and amino-acid side chains (Figure 1A) [1].

In PSII, primary charge separation occurs at the reaction center chlorophyll-a electron donor, P_{680} . The excited state, P_{680}^* , donates an electron to pheophytin, which in turn reduces the primary PQ electron acceptor, Q_A . Q_A^- reduces the secondary PQ electron acceptor, Q_B . After receiving two electrons and two protons, plastoquinol (Q_BH_2) diffuses out of its binding pocket and is replaced by an oxidized PQ molecule from the membrane-soluble PQ pool. At the donor side of PSII, the hole remaining in P_{680}^+ is filled by a redox-active tyrosine, Y_Z , which is subsequently reduced by the OEC.

At the OEC, O_2 is produced via a four-step stochastic mechanism in which each intermediate is produced following excitation of P_{680} . Dark-adapted PSII can undergo single turnovers using short saturating flashes of visible light. Joliot found that O_2 was generated in high yield after the third flash and every fourth flash thereafter [2]. Using these data, Kok and coworkers developed a model, now known as the Kok cycle, in which the OEC cycles through four light-generated intermediates known as S states [3]. As shown in Figure 1B, dark-adapted PSII poised in S_1 will be converted to S_4 following three flashes of light. Upon its formation, S_4 spontaneously releases O_2 and reforms S_0 .

While the chemistry of OEC turnover has received much attention, a mechanism of substrate water binding and O-O bond formation has yet to be generally agreed upon. Herein, we focus on the hydrogen-bond networks in the second-shell protein environment around the OEC that efficiently transport protons and O_2 out of the active site, while simultaneously allowing water access to the OEC. The naturally occurring residues that ligate the OEC (Figure 1A) are required for efficient photoassembly and O_2 evolution (reviewed in Ref. [4]). Protein mutants

have also determined that Y_z is D1-Y161 and shares a hydrogen bond with D1-H190 (reviewed in Ref. [5]). Water oxidation is not possible, or at best diminished, when any of these first-shell residues are mutated. Second-shell residues also play a key role in proton transport and tuning the properties of the OEC. In this review, we focus on the non-ligating protein environment of PSII within 15 Å of the OEC, in which mutations and channel perturbations have substantial effects on the hydrogen-bond networks.

Channels in Photosystem II

A number of water-filled channels in PSII protein structures can form long-range hydrogen-bonded networks. One series of connected voids in PSII crystal structures (named “i” [8], “back” [9], or A1/A2 [10]) was originally proposed to be a channel for water or O₂ transport. This channel has been shown via molecular dynamics to be impermeable to water [11], with a barrier of ~22 kcal/mol at the bottleneck composed of D1-L91, CP43-F292, and CP43-F358 [12]. This leaves the “narrow,” “broad,” and “large” channel systems as options for water entry from the lumen to the OEC (Figure 2, with nomenclature from [9,13,14]). The narrow channel connects to D1-D61 and the O4 μ -oxo ligand of the OEC and has a calculated 9 kcal/mol barrier for water entry at the interface of D1-N338, D2-N350, and CP43-P334 [12]. Just beyond this barrier, the channel reaches the lumen at the PsbO and PsbU interface in cyanobacteria (Figure 2B). On the other side of D1-D61, the broad channel passes the D1-E65, D1-R334, D2-E312 bottleneck (12 kcal/mol water barrier [12]), exiting at the interface of the D2 and PsbO subunits (Figure 2B). This channel is postulated to serve as a proton-exit pathway [15,16]. Finally, the large channel, with its many branches, is a possible route for delivering substrate water to the OEC (Figure 2B). The calculated activation barrier of 10 kcal/mol for water molecules occurs at the junction of pathways near D1-E329 [12], a residue affected by the S-state transitions [17]. The many studies that introduce point mutations or external inhibitors take advantage of these channels to modify the electrostatic or hydrogen-bonding environment surrounding the OEC.

Water substrate exchange

The exchange of ¹⁸O-labeled bulk water with substrate waters of the OEC is a direct experimental probe of the hydrogen-bonding networks involved in water transport [18]. The two substrate waters required for the generation of one O₂ molecule are referred to as the fast (W_f) and slow (W_s) waters based on their relative rates of exchange. Both W_f and W_s exchange rates have an inverse H/D isotope effect of 0.73 and 0.94, respectively [19]. Replacing H₂O with D₂O

strongly perturbs the hydrogen-bonding network in the case of W_f but hardly effects W_s , leading to the possibility that either the two substrates are delivered to the OEC via different water channels or there are multiple microscopic H/D isotope effects that can enhance or suppress the measured isotope effects of the two waters asymmetrically.

Mutations of the second-sphere amino acids around the OEC affect the ^{18}O water exchange rates, highlighting their role in substrate water transport. The D1-D61N mutation slows down both W_s and W_f exchange rates by 3 and 6.5 times, respectively [20]. This is especially surprising because mutations of some of the direct ligands to the OEC, such as D1-D170 and D1-E189, have less dramatic effects [18] indicating that hydrogen-bonding networks play important roles in water exchange kinetics.

A chloride ion, situated in the broad channel 6 Å away from the OEC, also regulates substrate water exchange. ^{18}O water exchange kinetics in PSII substituted with $\text{Sr}^{2+}/\text{Br}^-$ and $\text{Sr}^{2+}/\text{I}^-$ in place of $\text{Ca}^{2+}/\text{Cl}^-$ have shown that exchange of W_f is 1.5 times slower and W_s is 9.5 times faster in the S_3 state of PSII [21]. In contrast, in PSII substituted with only Sr^{2+} , W_f is unaffected while the W_s exchange rate increased by a factor of 3-4 [22]. The difference in the water exchange rates of Sr^{2+} substituted and $\text{Sr}^{2+}/\text{Br}^-$ or $\text{Sr}^{2+}/\text{I}^-$ substituted PSII can be attributed to changes in the hydrogen-bond network around the anion in the broad channel.

Y_Z electron transfer

Natural variations in the amino-acid sequence of D1 have been identified, mainly in cyanobacteria that express different D1 isoforms when grown under different conditions. While many of the alternate residues affect photoprotection and electron transfer [23], there are some substitutions in the region around the OEC that affect Y_Z electron transfer and progression through the Kok cycle. The inclusion of the *psbA2*-encoded D1 protein of *Thermosynechococcus elongatus* hinders the S_2 -to- S_3 transition [24], due to changes in the hydrogen-bond network around D1-P173M [25]. Site-directed mutation of D1-P173G in *Synechocystis* sp. PCC 6803 also shows decreased electron transfer from Y_Z to P_{680} and only 62% of wild-type O_2 evolution [26]. Point mutations based on D1 protein variants in *Synechocystis* sp. PCC 6803 show that the D1-F186L mutation results in only 70% of wild-type O_2 evolution, while D1-P162S has no observable effect [27]. Tellingly, both residues are next to D1-Y161, but only the backbone of residue D1-186 provides a hydrogen bond to the water network around the OEC (see Figure 3B).

Equilibrium between S_2 isomers

S_2 , generally agreed to be $Mn^{3+}(Mn^{4+})_3$, is the most accessible EPR-active state and is present as two isomers [28]. The $S = 1/2$ spin state is characterized by a multiline EPR signal at $g = 2$, while the $S = 5/2$ spin state shows a broad EPR signal at $g \approx 4.1$. As recently reviewed by Pokhrel and Brudvig, the specific hydrogen-bonding network around the OEC strongly affects which S_2 isomer is favored [29]. In PSII samples from higher plants with sucrose as a cryoprotectant, both isomers are present, but for wild-type cyanobacteria, only the $S = 1/2$ S_2 state is observed [29]. However, for point mutants in *Synechocystis* sp. PCC 6803, the $S = 5/2$ signal was detected in mutants D2-K317R [30], D1-A344G, D1-A344D, and D1-A344N [31]. PSII containing the D2-K317R mutation is still dependent on Cl^- (~ 6 Å from Mn4 [6]), but has lower steady-state O_2 -evolution activity and less efficient S-state turnover [30]. Presumably, the presence of D2-R317 not only decreases proton-release efficiency in the broad channel, but also propagates changes in the hydrogen-bonding environment directly surrounding the OEC. Mutants at D1-A344 likely affect the OEC environment more directly as the C-terminus of the D1 peptide that ligates Ca and Mn2 (Figure 1A) [6] following post-translational cleavage [32]. Changing the side chain of A344 is not likely to change OEC ligation, but would influence the surrounding hydrogen-bond network, which in turn may affect the relative populations of the two S_2 isomers.

The absence of the $S = 5/2$ S_2 isomer in wild-type cyanobacteria is not understood, so we analyzed the D1 sequences of cyanobacteria, algae, and plants for any differences that might account for the change in S_2 isomer populations. Between cyanobacteria and higher plants, the most significant variation near hydrogen-bonding networks is located at D1-87. The change from asparagine in cyanobacteria and green algae to alanine in higher plants will modify the shape and flexibility of the narrow channel, while also removing a hydrogen-bonding residue. We postulate that altering this hydrogen-bond network modulates the relative energy of the two S_2 isomers (Figure 3D).

Formation of the S_3 state

Because the S_1 -to- S_2 transition does not involve proton release, changes in the hydrogen-bonding network around the OEC should not cause large effects until the S_2 -to- S_3 transition (Figure 1B). The S_3 state, $(Mn^{4+})_4$, has recently been characterized by multidimensional EPR with each Mn center having octahedral coordination [33]. This implies that an additional ligand is incorporated during the S_2 -to- S_3 transition to convert five-coordinate Mn(III) to six-coordinate Mn(IV), although it remains to be determined whether this occurs at Mn1 or Mn4. In contrast to the local OEC changes observed for the S_1 -to- S_2 transition in state-

resolved X-ray diffraction studies [34,35], protein motion in response to the formation of S_3 is observable at 5.5 Å resolution [36]. The rearrangement of protein in the S_3 state must be accompanied by an adjustment of the hydrogen-bond network since many waters surrounding the OEC are stabilized by hydrogen bonds to backbone atoms.

Proton-release pathways

All S-state transitions except S_1 to S_2 involve proton release to the lumen (Figure 1B) as determined by pH dependence, H/D isotope effects [37], and photoacoustic spectroscopy [38]. The S_3 -to- S_0 transition occurs with a half-life of 1-2 ms [39] and is generally agreed to be the rate-limiting step of OEC turnover. While the OEC has a hypothetical maximum turnover frequency of $\geq 500 \text{ s}^{-1}$, the observed turnover rate of PSII is $25\text{-}88 \text{ O}_2 \text{ s}^{-1}$ (reviewed in Ref. [40]). This kinetic limitation of OEC turnover is often controlled by PQ exchange, but can also be limited by proton release to the lumen [41].

The broad channel is postulated to be a proton-release pathway and is lined by a number of hydrogen-bonding residues (Figure 3B). This water-filled channel includes the area around D1-N181 and D1-V185 and extends past a chloride ion, D1-D61, and D2-K317 before reaching residues on the lumenal surface. The calculated pK_a values for residues along the channel increase in the direction of the lumen, favoring proton transport away from the OEC [15]. Although the D1-E65, D1-R334, and D2-E312 residues form a 12 kcal/mol barrier for water transport [12], the two glutamate residues can share a proton and are postulated to be a “proton antenna” [16]. Experimental characterization shows that mutations D1-E65A [17], D2-E312A [17], D1-R334A [42], D1-D61A [43], and D2-K317A [30] or D2-K317R [44] affect carboxylate and amide FTIR features in similar ways. Based on FTIR data from other mutants, the hydrogen-bond network involved in proton release may even extend to D1-E329 [17] or D1-Q165 [42]. The side chain of D1-D61 clearly plays a role in stabilizing waters as the FTIR feature at 3663 cm^{-1} in the O-H stretching region for weakly hydrogen-bonded waters is eliminated by the D1-D61A mutant [43]. X-ray exposure results in 67% of the broad channel residues showing oxidative modification due to generation of reactive oxygen species (ROS) from waters in the channel [45].

O₂-release pathways

It remains unclear whether there is a dedicated route to facilitate the transport of O_2 away from the OEC. In standard spinach PSII preparations, oxidative modification of protein residues, which may be due to ROS chemistry, occurs only along the narrow channel (i.e. at

CP43: E354, T355, M356, and R357) [46] (Figure 3D). Derivatization of PSII crystals with Kr resulted in two Kr atoms bound in the large channel system suggested to be an O₂-exit pathway [10]. Subsequent computational simulations of O₂ diffusion show that the O₂ molecules generally follow the water pathways, with likely exit paths overlapping portions of either the large or broad channels [47,48]. Intriguingly, the D1-E329 residue that hinders water diffusion in the large channel poses no barrier for O₂ diffusion [47] (Figure 3C), providing a likely mechanism for PSII to control water access to the OEC but facilitate the exit of O₂.

Experimental determination of O₂-release kinetics is also useful to highlight possible O₂-exit pathways. For example, mutation of the D2-K317 residue in the broad channel results in slower O₂ release [30]. Also near the OEC, the D1-V185N mutant has the slowest measured O₂ release kinetics of any mutant [26]. It remains possible that these mutations also affect the chemical mechanism of O-O bond formation or the efficiency of proton release through the hydrogen-bond network rather than directly affecting O₂-diffusion kinetics. Therefore, it would be interesting to explore whether mutations along the large channel would affect O₂ release, although the branched structure of the large channel system may require careful choice of mutants to study. For example, in proteins created by random mutagenesis, both the single mutant D1-N322I and the double mutant D1-I326F/D1-F328S showed impaired growth in high light, but the triple mutant is phototolerant [49]. The slow-growing D1-L341P mutant [50], however, is an interesting candidate for further characterization.

Conclusions

The hydrogen-bond networks surrounding the OEC on the luminal side of the PSII protein serve to stabilize charged intermediates and regulate the structure of the channels that allow transport of water, protons, and O₂. While many residues lining the broad channel have been characterized, there are opportunities to probe the water network in the narrow and large channels. Amino-acid residues along these channels can affect the hydrogen-bond networks directly via side-chain interactions or by perturbing the location of the protein backbone. Furthermore, comparing the species- and growth condition-dependent differences in these regions of the PSII complex will help elucidate which residues are important for regulating the complicated water-oxidation chemistry.

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Figure Legends

Figure 1. (A) Structure of the OEC and coordinating residues from the 1.9 Å crystal structure [6] and computationally optimized for the S_1 state [7] showing Mn (purple), Ca (orange) and O (red). All labeled side chains are from the D1 residue, except CP43-Glu354. (B) The Kok cycle. Starting from the dark-stable S_1 state, electrons (blue) and protons (red) are released in an alternating pattern [3]. O_2 is released upon formation of the S_4 state.

Figure 2. (A) The water-filled channels around the OEC are shown in the context of the PSII monomer [6]. Subunits with residues that line the channels are highlighted in color: D1 and D2 in blues, antenna proteins in greens, and PsbO in purple. Water molecules shown in orange form the “narrow” [9,13,14] channel (also known as E/F [10], or channel 2 [12]). The yellow surface shows the water network corresponding to the channels termed “broad” [9,13,14], “iii” [8], D/C/G [10], or 1/3 [12]. In red is the branching network described as the “large channel system” [9,13,14], “ii” [8], B1/B2 [10], or 4a/4b [12]. (B) The water molecules in the 1.9 Å crystal structure are shown as spheres within each colored-mesh surface for the channels shown in panel (A) [6]. The large channel (in red) also contains two glycerol molecules introduced during crystallization. The protein environment around each channel is depicted as colored ribbons (D1: dark blue, D2: light blue, CP43: light green).

Figure 3. (A) The channel systems detailed in Figure 1B are shown without the protein environment. In panels B-D, important side chains and hydrogen-bonding backbone atoms are shown in licorice with the protein displayed as ribbons (D1: dark blue, D2, light blue, CP43: light green, PsbU/PsbV: gray). The OEC atoms are shown as connected spheres with Mn (purple), Ca (orange), and O (red). Important residues and water molecules that are first-shell ligands to the OEC are labeled in bold. Transparent red surfaces highlight residues along each channel calculated to form a barrier to water transport [12]. (B) The water molecules that form the broad channel are shown outlined in a yellow mesh. The location of chloride ion in the 1.9 Å crystal structure is shown as a green sphere [6]. (C) Many branches join to create the large channel (red mesh). Two glycerol molecules found in the crystal structure [6] are also included inside the mesh outline of the channel. (D) The narrow channel (orange mesh) extends past residues that show oxidative modification [47] and may link to another channel for water transport (channel X, not shown) found during molecular dynamics simulations [12].

Hydrogen-bonding channels in Photosystem II

Figure 1

Figure 2

Figure 3