

Structure and Catalytic Activity of the Oxygen-Evolving Complex of Photosystem II

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The oxidation of water in the thylakoid membranes of many photosynthetic organisms is the first crucial step in deriving chemical power from sunlight. This reaction occurs in the oxygen-evolving complex of photosystem II, employing a manganese-calcium metalloenzyme core for a catalyst. The structure of this cluster of atoms and the exact mechanism of photooxidation by the complex remains largely unknown, though recent data collected from X-ray spectroscopy, electron paramagnetic resonance spectroscopy, and fluorescence experiments hold promising results. As these are the first reaction and enzyme that link the radiation energy of the sun to the organic chemical energy of the biosphere, a complete understanding of the structure and pathway are essential to fully understanding biotic energy production on Earth.

I. INTRODUCTION

The evolution of photo-oxidation of water in early cells represented a dramatic shift in the energy capture of life on Earth. The earliest life forms were most likely chemotrophic, meaning that they extracted energy from the oxidation of inorganic minerals and chemicals such as H_2S , Fe^{2+} and Mn^{2+} . Using water as an essentially inexhaustible source of electrons, early phototrophic organisms quickly filled the atmosphere with O_2 , permitting the development of the vastly more efficient aerobic metabolisms in life forms that we see today.¹ Aside from the chemotrophs that still live in aphotic environments such as deep sea vents, all organisms on Earth now get their energy directly or indirectly from the radiant power of the sun.

This dependence on sunlight for biotic energy places an extreme importance on the mechanisms and enzymes responsible for facilitating photon energy capture and storage in photosynthesizing organisms. It is interesting to note that only one single class of enzymes are

found in all of the photosystem II (PSII) and oxygen-evolving complexes (OECs) studied in oxygenic phototrophs to date, from spinach to *Synechococcus*. Little variation in the PsbA gene that codes for the central protein binding the inorganic core is observed, and there is no variation in the $\text{Mn}_4\text{CaO}_x\text{Cl}_{1-2}(\text{HCO}_3)_y$ inorganic core itself. It seems that the past 3.8 billion years of evolution in all possible habitats permissive of photosynthetic metabolism have yielded only one biological blueprint for water splitting, a situation rarely seen in most biological pathways, wherein multiple mechanisms are derived to accomplish the same goal.¹⁻³

Although much is now known about the polypeptide and inorganic ion cofactor requirements for O_2 evolution, discovery of the mechanism of the O_2 -evolving reaction and the precise organization of its constituent parts still presents a formidable challenge to investigators working in the field of photosynthesis.⁴ Much of the pioneering work was done by B. Kok in 1970 when it was shown that O_2 evolution is a linear 4-electron oxidation reaction consisting of discrete oxidation states S_n with $n \in 0, 1, 2, 3, 4$.⁵ The first four of these transition states are fairly well understood, but the structure of S_4 is difficult to resolve. A complete picture of the entire Kok cycle will give us a great understanding of biological water-splitting, which may help improve our technologies to that effect, which currently require expensive catalysts and create damaging intermediates. By modeling what nature has tested for billions of years, we may find an economically viable solution to our current energy concerns.

II. THE OEC REACTION CYCLE: THE KOK CYCLE

The pathway for the photo-oxidation of water takes place at the inorganic core of the OEC. It has been well-established that water is split and oxidized in a five stage cycle known as the Kok cycle, as shown in Figure 2.⁵ The progression through each stage involves the absorption of a photon by PSII, creating a charge separation. The reaction center of PSII (P680) is a pair of chlorophyll *a* molecules coordinated by two protein subunits known as D1 and D2. The electrons generated by this charge separation are transferred first to pheophytin *a*, then the first quinone acceptor Q_A , and then the second quinone acceptor Q_B . On the oxidizing side of PSII, a tyrosine residue D1-Tyr-161 (Tyr_Z , Y_Z , or Z^+) reduces P680. The oxidized Y_Z withdraws electrons from the Mn cluster in the inorganic core of the OEC,

which in turn draws electrons from water, leading to a splitting of water and production of molecular oxygen.⁶ Figure 1 provides an overall view of PSII and the OEC.

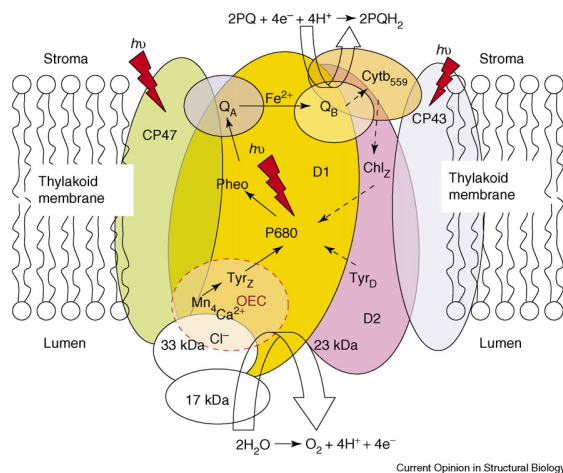


FIG. 1: Overview of PSII and OEC⁷

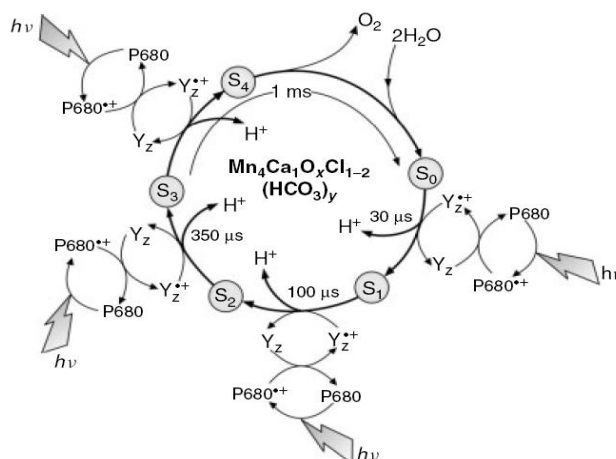


FIG. 2: Stages of the Kok Cycle⁵

The five stages of the Kok cycle are denoted S_{*i*} (as shown in Figure 2), where *i* represents the number of oxidative equivalents accumulated in the OEC. Only the S₁ state is stable over long periods, and within tens of minutes nearly all of the PSII centers in a prepared sample are in that state. The half-lives of the various state transitions are shown in Table I.⁴ The postulated S₄ stage is a transient intermediate that spontaneously returns to the S₀ state with the release of dioxygen. To fully understand the catalytic mechanism of photosynthetic oxygen evolution, it is essential to understand the structure of the Mn complex of the OEC at each S state.

TABLE I: Half-life ($T_{1/2}$) for the S-state transitions $S_i \rightarrow S_{i+1}$ ⁴

Reaction: $S_i \rightarrow S_{i+1}$	$T_{1/2}(\mu\text{s})$
$S_0 \rightarrow S_1$	30
$S_1 \rightarrow S_2$	100
$S_2 \rightarrow S_3$	350
$S_3 \rightarrow S_4$	1000
$S_4 \rightarrow S_0$	Very fast

III. DETERMINING THE STRUCTURE OF THE OEC

There are several experimental methods in determining the structure of the OEC and PSII during the stages of the Kok cycle. One such way to study the oxidation states and structural changes in the OEC is to use X-ray absorption spectroscopy (XAS) in conjunction with electron paramagnetic resonance spectroscopy (EPR). Extended X-ray absorption fine structure (EXAFS) provides information about the types, numbers, and distances of the neighboring atoms from the absorbing Mn atoms. Recent EXAFS and EPR data suggests that the Mn atoms are arranged in a “dimer of dimers” model for states S_1 and S_2 , such that two binuclear units are linked with a mono- μ -oxo-bridging ligand about 3.3 Å across.^{8,9} To resolve the structures in the S_0 and S_3 states, prepared samples are continuously illuminated at cryogenic temperatures to slow the reactive processes. Thus far, the S_4 state is too short-lived to study completely.¹⁰ Figure 3 shows the inferred structures of the Mn complex for the resolvable Kok stages based on EXAFS spectral data.

In addition to the Mn cluster, there are a few other cofactors required for oxygen production. EPR studies have indicated the presence of Ca^{2+} and Cl^- in the OEC in addition to manganese. The chlorine is thought to activate the Mn cluster in one of two ways: it may act as a charge-neutralizing counterion in the environment of the active site of the OEC, or it may bind to Mn or occupy a nearby site, facilitating electron transfer among the metal atoms.⁴ On the other hand, there is no available information on the binding location of the Ca^{2+} ions, though it is reasonable to assume that the site is on the interior of the structure of the supporting polypeptides, since reactivation of the OEC after ion depletion requires long-term incubation of calcium, in contrast to the immediate reactivation of the

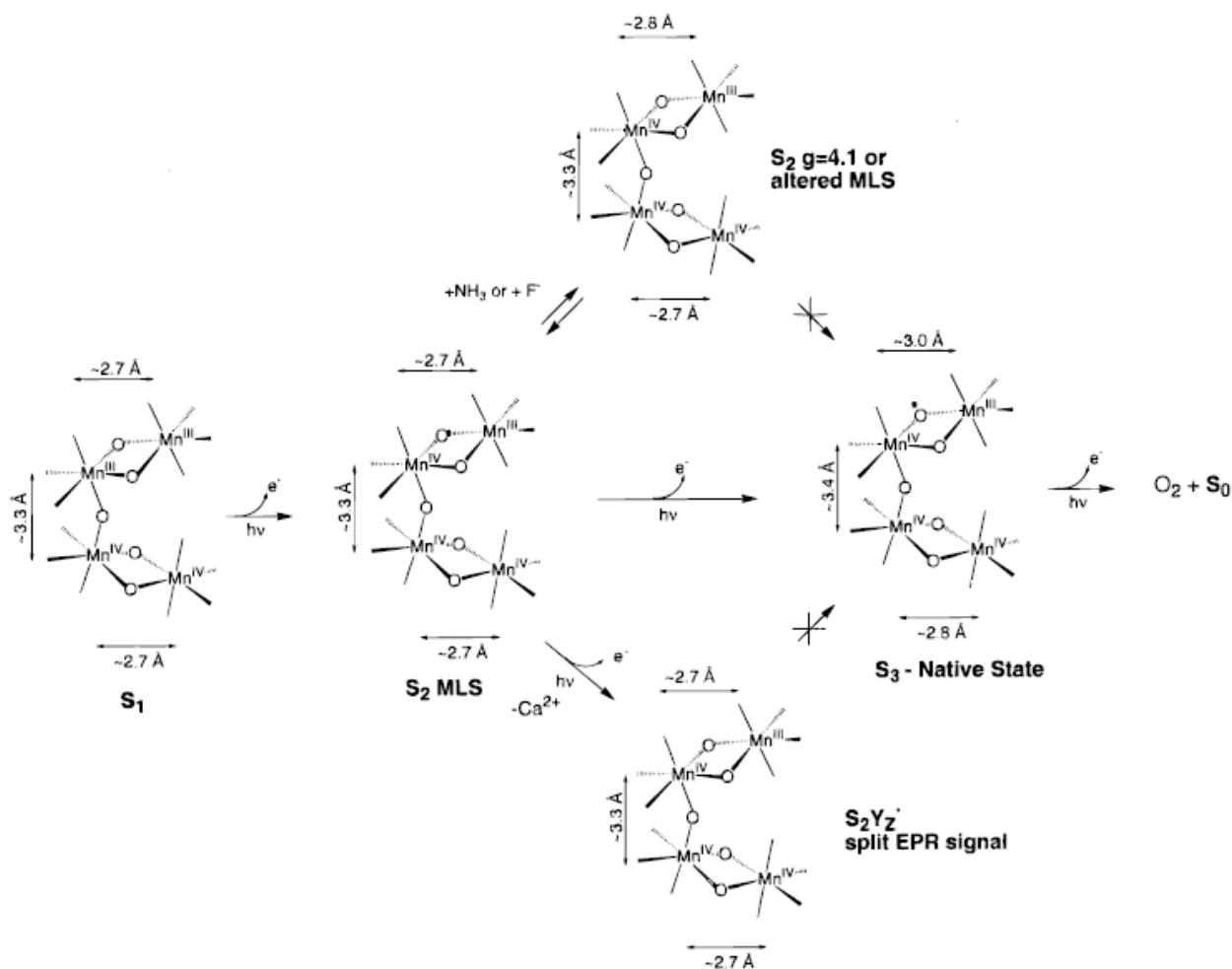


FIG. 3: Summary of the changes in Mn-Mn distances and oxidation states during the Kok Cycle S₁ to S₃⁸

protein-depleted complex upon addition of the ion.

The coordination of the polypeptides that surround the OEC are also crucial to continued catalytic activity. Aside from the necessary tyrosine residue for electron transfer to PSII, two histidine residues (H-190, H-332) on the D1 protein are needed for oxygen evolution. Without them, catalytic activity is lost, and it has been verified that H-190 is actually ligated to the Mn cluster, perhaps to facilitate proton conduction.¹¹ Binding of a proton released by water oxidation to one of the imidazole nitrogens could occur at the final S₄ → S₀ step of the Kok cycle as the nitrogen basicity increases with the re-reduction of manganese.¹² Figure 4 displays structural models of the OEC/PSII center described by (a) X-ray crystallography and (b) DFT QM/MM simulations, and we can see the proximity of the cofactors and active

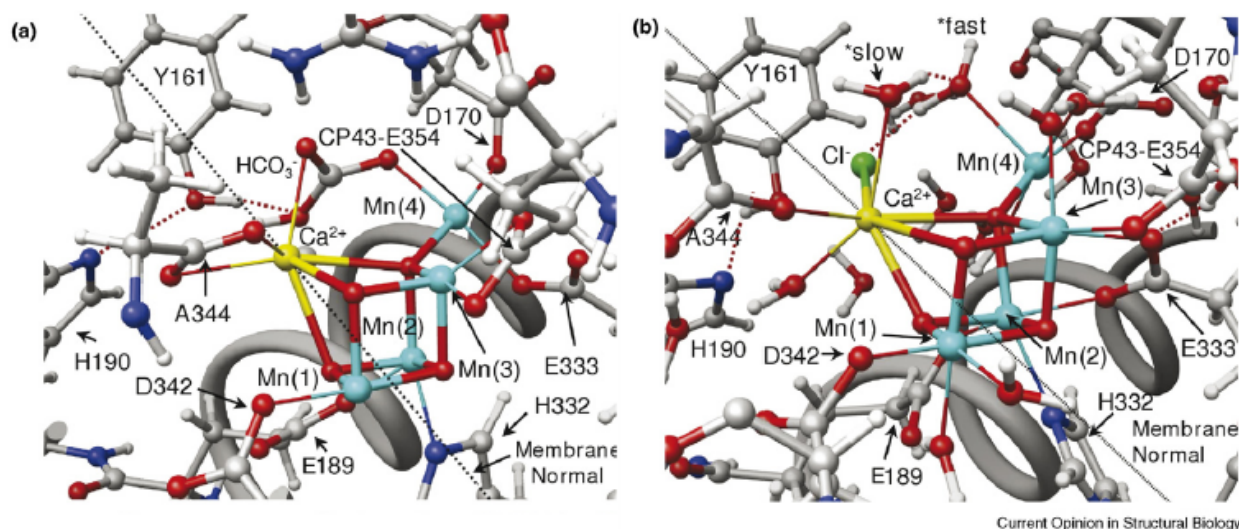


FIG. 4: Structural models of the OEC and PSII as described by (a) X-ray crystallography and (b) DFT QM/MM hybrid models⁷

amino acid residues to the manganese complex.

IV. DETERMINING THE MECHANISM OF OEC REACTIONS

Historically, EPR provided the first experiments to identify the coupled Mn cluster at the catalytic site, and recently EPR and XAS tools have become indispensable to characterize both the redox chemistry and atomic structure of the PSII/OEC core. EPR signals are now available for states S_0 through S_4 , and measurements of the Zeeman g -factor and zero-field splittings as well as ^{55}Mn hyperfine structure reveal that all of the S states are formed in low-spin ground electronic states. The kinetics and thermodynamics of each step have been measured by time-resolved UV-absorption changes of Mn, leading to the energy diagram shown in Figure 5. The rate limiting step is the last $S_3 \rightarrow S_0$ reaction, which we saw earlier in Table I.¹

The OEC water-splitting mechanism depends mainly on the electrochemistry of the inorganic core manganese atoms, but there is still a dependence on the protein structure in which this cofactor is embedded. As mentioned, two very important aromatic residues on the D1 polypeptide have been identified near the OEC core: the Tyr-161 and His-190 amino acids. Also of importance is the dark-stable residue Tyr-160 on D2 (D^+). These seem to be the important charge carriers for moving electrons and protons out of the OEC

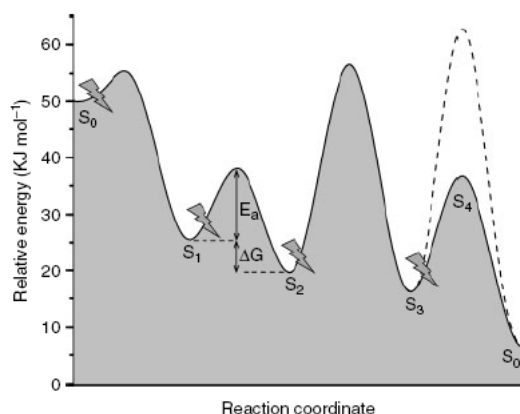


FIG. 5: Free-energy change over the Kok cycle. The dashed line is a higher energy transition that exists for lower temperature measurements¹

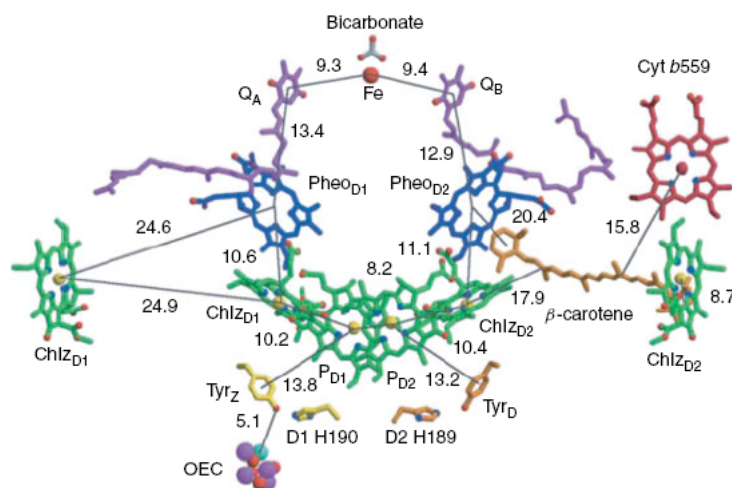


FIG. 6: XRD model of cofactors bound to PSII/OEC. View is perpendicular to membrane normal¹

Mn complex so that the cycle may continue, as well as shuttling the reducing electrons to quinones and other intermediates for PSII to use.¹³ Experiments have also confirmed that the mechanism of oxygen-evolution in PSII are highly sensitive to temperature changes, and fluorescence spectroscopy data have determined that above 40° C the activity of the OEC drops off rapidly, with PSII losing nearly all charge separation ability above 50° C.¹⁴ Figure 6 shows the relative spatial arrangement of important cofactors for the OEC and PSII as a whole.

V. CONCLUSIONS

While much of the structure and mechanism for oxygen evolution in the OEC has been determined, there is still work to be done, mainly with the highly transient S_4 stage of the Kok cycle, though some mechanisms are postulated for the formation and release of oxygen in this step.¹ Table II summarizes the current opinion regarding the stoichiometries and functions of essential components of PSII and the OEC. The organic and inorganic catalysts of photochemical oxygen evolution by PSII are ligated to a template comprised of several polypeptides. The evolving process is one in which 3 or 4 oxidizing equivalents are stored as oxidation-state advancements in Mn; the fourth oxidation-state advancement within the OEC leads to the 4-electron oxidation of H_2O . Although Ca^{2+} and Cl^- are not required for Mn oxidation, omission of either of these essential cofactors blocks water oxidation, so these ions are necessary elements in maintaining the active structure of the Mn ensemble.⁴

TABLE II: Essential components of the OEC and PSII⁴

Cofactor	Stoichiometry	Function	Polypeptide(s) involved in cofactor ligation ^a
Mn	4	H_2O oxidation	?(47, 43, 33, D1, D2)
Ca^{2+}	2-3	regulation of Mn function in H_2O oxidation	?(47, 43, 33, D1, D2, 23)
Cl^-	4-5(?)	"	"
PQ	2	electron acceptors (Q_A and Q_B)	34(D2), 32(D1)
Chl <i>a</i>	50	photochemistry/antenna	47, 43, D1, D2
Pheophytin <i>a</i>	2	charge separation	34(D2), 32(D1)
D^+	1	oxidation of S_0 to S_1	34(D2)
Z^+	1	oxidation of Mn/reduction of $P680^+$	32(D1)
Non-heme Fe	1	regulation of Q_A/Q_B electron transfer	D1, D2
Heme Fe	2(<i>b559</i>)	?	9, 4.5

^aThe numbers given are the estimated molecular masses in kDa

The current state of knowledge about PSII and water oxidation defines the obvious challenges that must be overcome to arrive at a complete understanding of the photosystem

and its most important function. The molecular identity and structure of cofactor binding sites must be uncovered and the interactions among the many essential polypeptides must be elucidated. The way in which polypeptide structure regulates oxygen evolution activity also remains a mystery. Recent work has shown that the structures required by plants for photosynthesis are polypeptides that can be manipulated like any other, so that further testing of more subtle reactions may be performed in the future.

VI. ACKNOWLEDGEMENTS

The works of Ghanotakis, Dismukes, and Kok^{1,4,5} were most helpful to me in fully understanding the reaction pathways and overall structure of the oxygen-evolving complex and its interaction with PSII and the rest of the photosynthetic process. They provided a thorough overview of the history of research on the subject as well as current work.

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