

The Oxygen Evolution Center in Photosynthesis

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Oxygen, which is essential to all animal life on earth, is produced by plants by the oxidation of water. This process is catalyzed by a tetranuclear manganese enzyme known as the oxygen evolution center [1]. The oxygen evolution center is present in the part of the photosynthetic apparatus known as photosystem II and is believed to reside near the reaction center. The enzyme is believed to contain three polypeptides (17, 23, and 33 KDa) [2] all of which are necessary for oxygen production. In addition to manganese and the peptides, both calcium [3] and chloride [4] are essential cofactors, although the roles of these latter ions are unclear [5].

The catalyzed reaction can be summarized as follows:



In the oxidation of water to oxygen, the electrons are transferred to the reaction center (starting at P680-the special pair). The protons diffuse toward the interior of the thylakoid membrane where they assist in the formation of ATP. From the plant's viewpoint, oxygen is a waste-product.

Pioneering mechanistic studies of water oxidation were carried out by Kok [6] and coworkers. Kok examined the maximum yield and rate of oxygen evolution as photosystem II was successively exposed to flashes of light. It was found that dark-adapted chloroplasts, initially in the resting state known as the S_1 state gave a maximal response after the third flash of the first cycle and after the fourth flash thereafter. These results led to the proposal of the reaction cycle known as the S state mechanism (Figure 1.)

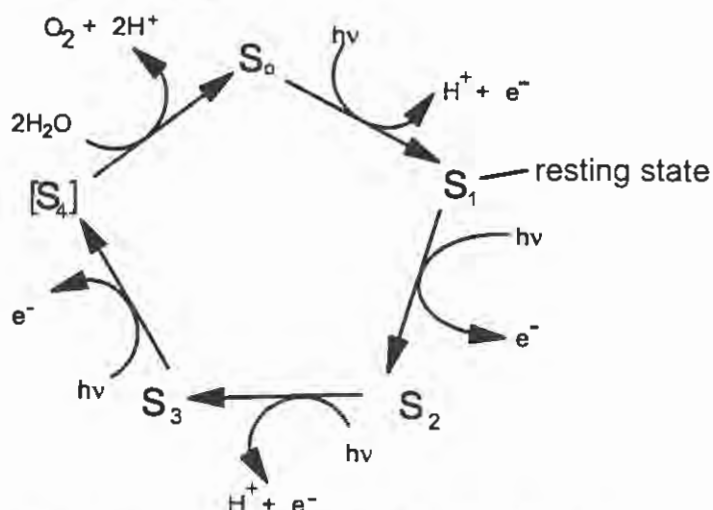


Figure 1. Model for charge accumulation - the S cycle.

From the mechanism we see that the oxidation of water to oxygen does not occur until after the fourth flash.

The structure of the enzyme responsible for water oxidation still remains an enigma [7]. X-Ray absorption spectroscopy (XAS) has been used to determine the oxidation states

and local environments of the manganese centers by comparison with XAS data for model manganese compounds [8]. The EXAFS (Extended X-ray absorption fine structure) portion of the spectrum has revealed the presence of several Mn-Mn and Mn-ligand interactions [9]. The presence of calcium at the active site has been confirmed by changes in the EXAFS spectrum upon treatment of the enzyme with Sr^{2+} [3a]. The XANES (X-Ray absorption near edge spectrum) portion of the spectrum reveals a change in oxidation state upon going from the S_0 to S_1 and from the S_1 to S_2 states. However, XANES data indicate that no metal centered oxidation occurs during conversion from the S_2 to S_3 state [9d]. It has been implied from XANES data that the oxidation state distribution is (II)(III)(IV)₂ for S_0 and (III)₂(IV)₂ for S_1 .

Electron paramagnetic resonance spectroscopy (EPR) [10] has been employed to examine the paramagnetic intermediates in the S cycle. It has been confirmed, by flash experiments [11], that the S_2 state is paramagnetic. Two signals have been observed for this state: a multiline signal [10] centered at $g = 2$, and a $g = 4.1$ signal [12] with a line width of 320G which has been "trapped" at low temperature. Model compounds have assisted in the interpretation of the multiline signal [13].

The $g = 4.1$ signal converts to the multiline signal when the samples are warmed to 190K. Ammonia stabilized samples show complex hyperfine structure which implies a multinuclear origin for this signal. The ground spin states of the manganese centers responsible for the two signals are 1/2 and 5/2, respectively. The signal is attributed to a conformationally induced change in the spin state of the tetranuclear manganese cluster. EPR spectra of Ca^{2+} depleted samples reveal that a histidine residue is oxidized [14] during the S_2 to S_3 transition, a conclusion that is consistent with the XANES data. Figure 2 shows the proposed structure of the complex.

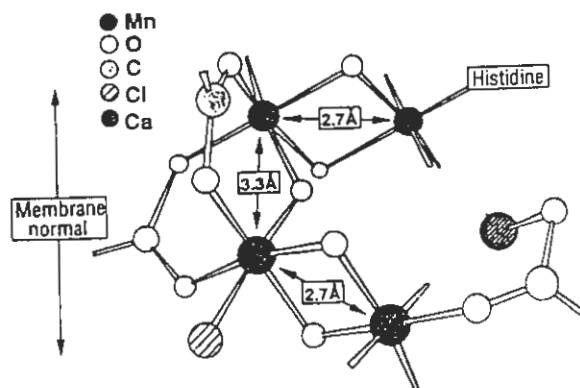


Figure 2. Proposed model for the active site of the OEC.

The structure [9A] is consistent with both EXAFS and EPR data. It can be viewed as a dimer of dimers which can exist in a variety of oxidation states as the enzyme catalyzes the oxidation of water.

The binding or presence of nitrogenous ligands and amino acids has been examined by a pulse EPR technique known as ESEEM [15] (electron spin echo envelope modulation spectroscopy), which can measure super hyperfine and quadrupolar interactions. The ESEEM data confirm that ammonia binds [16] to manganese in the S_2 state. Furthermore, the magnitudes of the quadrupolar couplings have implied that ammonia is deprotonated and exists as an amido species. Finally ESEEM data confirm (by isotopic labeling) that nitrogenous amino acids [17], such as histidine, are directly ligated to manganese.

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