A Functional Model of the O–O Bond Formation by the O₂-Evolving Complex in Photosystem II


Condensation and commentary by Juan C. Noveron and Pradip K. Mascharak, University of California, Santa Cruz

Condensation of the Research

Purpose of the Study
To investigate the structure of the active site that converts water to dioxygen (O₂) at the manganese cluster in Photosystem II, also known as the oxygen-evolving complex in photosynthesis

Background
Most life forms require dioxygen (O₂) to maintain the essential processes of life. The accumulation of dioxygen in the atmosphere has been attributed to plants and algae that catalyze the oxidation of water to dioxygen (Reaction 1) during a process called photosynthesis.¹

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2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-
\] (1)

This process takes place at a set of two protein assemblies called Photosystem I and II (PS I, II) located in the thylakoid membrane of chloroplast (Fig. 1). Together, they orchestrate an elaborated redox cycle called photosynthesis, in which light and water are used to produce ATP, H⁺, NADPH, and O₂.² The process begins when chlorophyll pigment molecules at the chlorophyll complex (P680) in PS II absorb light via excitation of their electrons. The excited electrons are then transferred to soluble electron carriers (Ph, PQ)³ that deliver them to PS I for the production of ATP and NADPH. The initial loss of electrons at the chlorophyll complex (P680) results in a charge separation between the components (referred P680⁺) with a very large redox potential (~1.80 V). A manganese cluster that is localized in a protein subunit near P680 catalyzes the oxidation
Figure 1. Schematic arrangement of the protein PS II in the thylakoid membrane of chloroplast. Chl = chlorophyll and Pheophytin and Qx are soluble electron carriers. The manganese cluster is the oxygen-evolving complex (OEC) of Photosystem II. (Adapted from Frausto da Silva, J.J.R., Williams, R.J.P. The Biological Chemistry of the Elements, Clarendon: Oxford, 1991.)

of water to dioxygen (Reaction 1) and transfers the resulting 4e\textsuperscript{−} to the chlorophyll complex P680\textsuperscript{+} to regenerate its ground state (P680). The manganese cluster in PS II has been named the oxygen-evolving complex (OEC) because oxygen is generated at such site during photosynthesis. To date, the chemical events that lead to the oxidation of water to dioxygen by the manganese cluster in PS II remain unknown. The chemical mechanism of OEC is of fundamental importance in science, not only for the implication that it has for life on Earth, but also for the chemical marvel that it possesses.

Recent EXAFS\textsuperscript{4} data have indicated that the OEC in PS II is composed of a tetranuclear cluster of manganese ions configured in a dimer of dimers.\textsuperscript{5} The two di-\(\mu\)-oxo-bridged manganese dinuclear units with a Mn–Mn separation of 2.7 Å are linked together by one mono-\(\mu\)-oxo and one mono- or dicarboxylato bridges with a Mn–Mn separation of 3.3 Å as shown in Figure 2. It is proposed that the manganese ions at the ground state (called S\textsubscript{0}) of the OEC exist as one Mn(II), one Mn(III), and two Mn(IV) centers. In addition, recent ESEEM\textsuperscript{4} results on \(^{15}\text{N}\)-grown OEC samples indicate the coordination of at least one histidine residue to one terminal manganese ion.\textsuperscript{6}

Spectroscopic investigations on the OEC in PS II have led to the observation of five intermediate states, S\textsubscript{0}–S\textsubscript{4}, during the oxidation of water to dioxygen.\textsuperscript{7} The intermediate states S\textsubscript{0}–S\textsubscript{4} of the OEC refer to accumulated oxidizing equivalents that involve structural rearrangements among the manganese centers accompanied with changes in their oxidation states. Dioxygen is released during the transition of S\textsubscript{4} to S\textsubscript{0} (Fig. 3). To date, several manganese complexes have been synthesized as mechanistic models of the OEC and studies on these synthetic analogues of the various S-states have shed some light into the nature of the different intermediates S\textsubscript{0}–S\textsubscript{4}. 

MAY 2000 PHOTOSYSTEM II, O\textsubscript{2}-EVOLVING COMPLEX, FUNCTIONAL MODEL 327
Figure 2. Schematic representation of the structure of the oxygen-evolving complex as suggested by the EXAFS data.\textsuperscript{5}

Figure 3. The five intermediate states (S\textsubscript{0}–S\textsubscript{4}) of OEC catalytic cycle.
Elucidation of the nature of the active species in the S₄ state of OEC that oxidizes water to dioxygen is of great importance in this field. It has been proposed that the S₄ state of OEC contains either a terminal Mn(V) = O⁹ unit or a dimanganese μ-oxo structure.⁹,¹⁰ The terminal hypervalent oxo species Mn(V) = O has received more attention recently since a synthetic ruthenium analogue with a Ru(V) = O moiety has been shown to oxidize water in a way similar to the OEC. However, most manganese complexes reported so far are either coordinatively saturated or lack structural features necessary to support such an active species. In the recent work of Crabtree and co-workers, the paper under review, the researchers synthesized the first multinuclear manganese complex containing a di-μ-oxo bridge and a labile site to hold a terminal oxo function. They also showed that such a complex mimics the reactivity of the S₄ state of the OEC of Photosystem II.

Researchers’ Approach

Crabtree and co-workers synthesized a structural analogue of the OEC of PS II that could generate a structure similar to the S₄ state of OEC. The complex is a dinuclear manganese species in which each Mn center is ligated to two bridging oxygens, three nitrogens from a terpy frame, and a labile water molecule. This model compound was isolated as a mixed valence Mn(III)–Mn(IV) dimer and was characterized by X-ray crystallography (Fig. 4). The researchers designed the model with labile sites so that it could generate the active Mn(V) = O species as postulated for the S₄ state of the OEC. They utilized an oxo-transfer reagent called sodium hypochloride (NaClO) to generate the hypervalent oxo species of 1 and then proceeded to investigate its chemical reactivity toward water, the natural substrate of OEC.

What Researchers Accomplished

The researchers were able to synthesize a model complex that possesses structural features and parameters similar to those noted with the OEC in PS II. For example, complex 1 contains a di-μ-oxo bridge between the

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Figure 4. Structure of the model complex 1.
manganese centers that results in a Mn–Mn distance of 2.7 Å, exactly the same distance postulated for the manganese centers in OEC by the EXAFS results. It also has one exchangeable water molecule on each Mn ion and thus allows for further reactivity. Moreover, the manganese centers in 1 exist in the mixed valence state of Mn(III)–Mn(IV) and mimics portion of the manganese cluster proposed for the ground state of the OEC. These similarities are presumably responsible for the first observation of successful O₂ evolution from water by a manganese dimer.

The reactions that lead to the formation of O₂ from water by 1 have also been identified by the researchers. When 1 reacts with aqueous sodium hypochlorite (an oxo-transfer reagent), a hypervalent manganese(V) oxo species is generated in solution. This active species exhibits absorption at 480 nm. This active intermediate with Mn(V) unit oxidizes water to dioxygen. The researchers reported that dioxygen evolved at a rate of 12 mol h⁻¹ per mol of 1 in a catalytic fashion (4 turnovers) at pH 8.6 and at room temperature and normal pressure (Fig. 5). The evolution of O₂ from the reaction was followed over time and it ceased 6 hours later when the active species was converted into permanganate.

The present study demonstrates that the hypervalent manganese-oxo intermediate generated in situ does have the capacity of converting water to molecular oxygen much like the OEC of PS II. The researchers have also provided a working hypothesis for this conversion. By using isotopically labeled H₂O¹⁸ and the technique of mass spectrometry, they have shown that all the dioxygen that evolves during the reaction originates from the solvent, i.e., one obtains mostly ³⁶O₂. This complete incorporation of ¹⁸O to dioxygen indicates that a hypervalent manganese-oxo species [Mn(V) = O] and not the hypochlorite (ClO⁻) is the oxidizing agent during the catalysis since the former is known to exchange with H₂O¹⁸ very rapidly (Fig. 6). Hypochlorite alone does not exchange with H₂O¹⁸ at a sufficient rate under the reaction conditions.

Finally, the researchers have provided a mechanism for the oxidation of water to molecular oxygen by the model complex 1. This mechanism (Fig. 7) involves the formation of a Mn(IV)–Mn(IV) dimer by
hypochlorite in the first step. Further reaction of hypochlorite with the Mn(IV)–Mn(IV) dimer generates the hypervalent manganese-oxo species. Subsequent reaction of the metal-oxo species with hydroxide results in the oxidation of water to dioxygen with concomitant formation of the Mn(III)–Mn(III) version of 1. The Mn(IV)–Mn(IV) dimer is regenerated in the reaction mixture due to the presence of excess hypochlorite and the cycle continues (Fig. 7). Since the model system exhibits no electron paramagnetic resonance (EPR) signal during catalysis, it is evident that the intermediates are Mn(IV)–Mn(IV) and/or Mn(V)–Mn(V) species as shown in Figure 7.
Commentary on the Research

This research illustrates how small synthetic analogues could provide insight into the fundamental aspects of highly complex systems. The researchers were able to show that a hypervalent manganese-oxo species can indeed react with water to produce dioxygen. This success strongly supports the notion that the active site of the OEC in Photosystem II contains a hypervalent manganese-oxo moiety. The ability of the model system to oxidize water to dioxygen under conditions applicable to living systems (i.e., pH 8.6, STP, mild oxidants) is truly remarkable. Although the model system evolves dioxygen at a much slower rate, it is the first example of a model system that mimics the functional aspects of the OEC of photosynthesis.

References and Notes

3. Soluble electron carriers: Ph, pheophytin; PQ, plastoquinones.
4. EXAFS, extended X-ray absorption fine structure: Measures interference of the photoelectron waves emanating from the metal center of a coordination unit with the waves back-scattered from the surrounding donor atoms. Such measurements provide information on the type, number, and distances of atoms in the first coordination sphere of the metal centers. ESEEM, electron spin-echo envelope modulation: Measures the coupling between electron(s) of the paramagnetic metal center and the nuclear spin moment(s) of nearby atoms. It provides information about neighboring nuclei weakly coupled to a metal center bearing electron spin density.