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Insights into the metalloradical mechanism of water oxidation

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Introduction

Photosynthetic water oxidation occurs at an active site comprising four manganese atoms and an oxidizable tyrosine residue. Six years ago, Gerald T. Babcock (Babcock, 1995) and coworkers (Tommos et al., 1995; Hoganson et al., 1995) developed a new hypothesis to describe the mechanism by which photosynthetic water oxidation might occur. During the ensuing six years, in response to continuous feedback from the scientific community, our chemical understanding has increased and we have refined the model so as to provide a clearer picture of the mechanism (Hoganson & Babcock, 1997, 2000; Tommos & Babcock, 1998, 2000; Tommos et al., 1998). The goals of this paper are to describe the essential features of the mechanism as currently understood, to describe recent tests of the model and to discuss some thermodynamic aspects of photosynthetic water oxidation.

Prior to 1995, the importance of P680 as the primary photo-oxidant of PSII, and of the four manganese atoms as essential for the catalytic oxidation of water had been established. Tyrosine 161 (Y_Z) of the D1 polypeptide was known to have the crucial role of passing reducing equivalents from the manganese to $P680^+$. It was widely believed that Y_Z donated electrons to $P680^+$ and extracted electrons from a tetranuclear manganese cluster, which functioned as a charge storage center (e.g., Babcock et al., 1989). New experimental and theoretical insights from several areas of chemistry and biochemistry forced a reevaluation of the role of Y_Z.

Tyrosine 161 was proposed to oxidize the manganese complex via hydrogen atom transfer reactions rather than by electron transfers (see also Gilchrist et al., 1995). The most important evidence in this regard was the finding from electron paramagnetic resonance spectroscopy that Y_Z and the manganese complex are less than 1 nanometer apart. This new role for Y_Z necessitated that during its oxidation by $P680^+$, protons should be lost rapidly from Y_Z and transferred ultimately to the aqueous phase. Histidine 190 of D1, hydrogen-bonded to Y_Z, was proposed to serve as the first acceptor of protons from Y_Z. The manganese complex thus lost its identity as a charge storage center, and its S-state cycle is now conceived of as being essentially electrically neutral.

We conceive of the manganese complex as losing hydrogen atoms from water molecules bound as terminal ligands to two of the four manganese atoms. Each loss of a hydrogen atom thus advances the substrate ligands successively from aquo to hydroxo, and ultimately, to oxo. The first three oxidations of the cycle are characterized as manganese-centered, with the electrons lost from the contingent of unpaired metal d-electrons, while the protons are lost from ligands to the manganese. The fourth oxidation is not stabilized at manganese, but instead results directly in O_2 formation and release. Oxygen-oxygen bond formation occurs only during the transition from S_3 to S_0. A crucial function of the manganese complex is to bind the two substrate ligands in a geometry that will promote O-O bond formation.

The formation of the oxygen-oxygen bond was proposed to be a radical reaction that requires direct participation of the tyrosyl radical $Y_Z^{\bullet}$. A concerted reaction is envisioned in

which a manganese-bound substrate OH is attacked simultaneously by electrophilic $Y_Z^\bullet$ (which abstracts the hydrogen) and by the electron-rich oxygen of a $O=Mn^{IV}$ species. Thus, the formation of the O-O single bond utilizes two one-electron oxidants, $Y_Z^\bullet$ and Mn(IV).

The functions of the essential calcium and chloride ions are less clear. Chloride may have a role in directing one or more protons of the manganese complex into locations suitable for abstraction by $Y_Z^\bullet$. This function could involve, during the S_1 to S_2 transition, a migration of the chloride from one binding site to another. The calcium ion, in addition to having a structural role, may assist in binding chloride, particularly during the lower S-states.

Experimental and theoretical tests of the proposed mechanism

Babcock, Tommos, Hoganson and coworkers have written extensively, describing and explaining the proposed mechanism of water oxidation. Here only a few of the results that test the proposed metalloradical mechanism are considered.

Hillier and Wydrzynski (2000) have shown that the substrate oxygen atoms are exchangeable in all the stable S-states and that the exchange rates vary with S-state. Di-μ-oxo bridged manganese dimers are likely present in PSII and, although they have been suggested as a precursor to molecular oxygen, the measured oxygen exchange rates are fast enough that the latter possibility can now be excluded. Terminal ligation of substrate oxygen is strongly favored instead. The exchange rate of one substrate oxygen is slow enough to be measured in all of the S-states, which suggests that it binds in S_0 and remains bound throughout the cycle. In S_3, the two oxygen atoms have distinct exchange rates; this provides strong evidence that the oxygen-oxygen bond is not yet present in S_3.

The variation of exchange rates with S-state is most simply explained as binding of the substrate water molecules to two distinct, redox-active manganese atoms. Monotonic decrease of the exchange rates does not occur, however, and an interplay between protonation state, coordination number, and ligand geometry may be needed to explain the observations.

The reactivity of terminal ligands has been demonstrated by studies of a model $Mn_2(\mu\text{-}O)_2$ complex constructed so that it can bind one terminal water ligand on each manganese (Limburg et al., 2001). The complex efficiently catalyzes oxygen evolution from reactive oxygen atom donors such as $KHSO_5$ and NaOCl. Oxygen nuclei from solvent water are incorporated into the product O_2. By contrast, $Mn_2(\mu\text{-}O)_2$ complexes that already provide a full ligand set do not readily produce molecular oxygen under strongly oxidizing conditions.

Oxygen-oxygen bond formation

The proposed metalloradical mechanism for O-O bond formation was originally explained by drawing analogy to the radical reactions of organic chemistry. Now several additional and more relevant reactions from inorganic chemistry can be mentioned, giving a more satisfying description of the crucial step in O_2 formation.

The S_3 state, only one hydrogen atom away from releasing O_2, has thermodynamic properties of an oxygen atom donor. This reactivity is most likely bestowed by the presence of a weak Mn-O bond (see below), such as may bind a terminal oxo ligand to a Mn(IV) atom, or as some similar structure. The weakness of the Mn-O bond makes it an effective radical trap. The presence of this radical trap together with $Y_Z^\bullet$ in the same active site allow a concerted reaction of these two species with an additional substrate OH group to form the new O-O bond.

Radical trapping reactions are exhibited by MnO_4^- and CrO_2Cl_2 . Carbon-centered radicals react with these reagents at diffusion-controlled rates, in part, by adding to the doubly bonded oxygen ligand, thereby reducing the metal by one electron (Steenkin & Neta, 1982; Nugent & Mayer, 1988; Mayer, 1998; Wistuba et al., 1999; Limburg, 2000; Strassner & Houk, 2000). Similar radical-trapping reactions participate in the "rebound mechanism" of cytochrome

$$4\ Y_Z^{\bullet} + 4\ H^{\bullet} \longrightarrow\ 4\ HY_Z \qquad\qquad (-86.5) \times 4$$

$$2\ H_2O \longrightarrow\ 2\ OH^{\bullet} + 2\ H^{\bullet} \qquad\qquad (+119) \times 2$$

$$2\ OH^{\bullet} \longrightarrow\ 2\ {}^{\bullet}O^{\bullet} + 2\ H^{\bullet} \qquad\qquad (+102) \times 2$$

$$2\ {}^{\bullet}O^{\bullet} \longrightarrow\ O_2 \qquad\qquad\qquad\qquad\quad -119$$

$$4\ Y_Z^{\bullet} + 2\ H_2O \longrightarrow\ 4\ HY_Z + O_2 \qquad -23\ \ kcal/mol$$

$$S0 + Y_Z^{\bullet} \longrightarrow\ S1 + HY_Z \qquad\qquad\qquad -7$$

$$S1 + Y_Z^{\bullet} \longrightarrow\ S2 + HY_Z \qquad\qquad\qquad -2$$

$$S2 + Y_Z^{\bullet} \longrightarrow\ S3 + HY_Z \qquad\qquad\qquad -2$$

$$S3 + Y_Z^{\bullet} \longrightarrow\ \text{"dry"}S0 + HY_Z + O_2 \qquad -4$$

$$\text{"dry"}S0 + 2\ H_2O \longrightarrow\ S0 \qquad\qquad\qquad -8$$

P450 (Harris et al., 2000), and are seen in compounds designed to be mimics of that enzyme (Lange et al., 1999). In cytochrome P450, it is an Fe^{IV}-OH group to which the organic radical adds, demonstrating that a doubly bonded ligand is not essential for radical trapping. The doubly-bonded nitrogen ligands of a chromium(VI) imido complex also trap organic free radicals (Chan & Nujent, 1985).

The previous examples all involve carbon radicals, but evidence for analogous reactions of oxygen radicals exists. Permanganate ion (Rüttinger & Dismukes, 1997) and bis(cyclopentadienyl)Mo=O (Silavwe et al., 1988) both release O_2 upon illumination. These reactions start with a ligand-to-metal charge transfer excitation, which converts an oxo ligand to a radical oxyl ligand. The oxyl radical then can add to a normal oxo ligand to make the new O-O single bond. Thus, considerable chemical precedent supports the plausibility of a radical mechanism of O-O bond-formation in PSII, as do theoretical considerations (Siegbahn & Crabtree, 1999).

Thermodynamic aspects: Mn-O bond strengths

The hypothesis of electroneutrality of the manganese complex enabled investigators to shift their thinking from reduction potentials of the S-state intermediates to bond strengths. To understand this, consider that if the manganese cluster is assumed to be electrically neutral, its interactions with its surroundings will be smaller than if it were charged—ions are always more strongly solvated than are neutral molecules. The solvation energy of the cluster will then be largely independent of S-state, since the changes of dipole moments and polarizabilities are likely to be small. In that case, the energetics will be dominated by the changes in bonding within the cluster, and not by interactions between the cluster and its surroundings. Although to neglect S-state dependent solvation is an approximation, it forms the rationale for focusing on the O-H bond strengths of the manganese ligands. We may pursue this idea by extending the focus to the Mn-O bond strengths. The structure of the complex affects the Mn-O bond strengths directly and, through them, the related O-H bond strengths.

Our previous analysis of the energetics of water oxidation by $Y_Z^{\bullet}$ utilizing standard bond strengths indicated the reaction to be weakly exothermic. (See top half of inset; for clarity, the phenolic hydrogen of tyrosine is retained in the symbol HY_Z). Others have suggested that the proton released from $Y_Z^{\bullet}$ should remain nearby. That would make $Y_Z^{\bullet}$ a stronger oxidant, but no extra oxidizing strength is needed.

We partition the enthalpy change of the complete S-state cycle into enthalpy changes for each transition between S-states and make quantitative estimates for each step. This is made possible by the experimental work of Vass and Styring (1991) who estimated the changes in reduction potential associated with S-state advancement from S_0 through S_3. These are directly related to free energy changes. Because we assume these S-state transitions to be

hydrogen atom transfers, they should involve very little entropy change: the enthalpies may be assumed to be similar in magnitude to the free energy changes. Values for the S_3 to S_0 transition can be filled in with educated guesses, backed by knowledge of energetics for water binding to manganese complexes from density functional modeling studies (Blomberg & Siegbahn, 1997).

Hess's law cycles provide the means to estimate the Mn-O bond strengths after oxidation by $Y_Z^{\bullet}$. We use standard bond strengths and the PSII enthalpies shown above (Hoganson & Babcock, 2000). The homolytic Mn-hydroxo bond energies are 44 kcal/mol in S_1 (see inset) and 39 kcal/mol in S_2, and the Mn-oxo bond energy in S_3 is 62 kcal/mol. These bond strengths can be used to indicate what intermediates and side reactions are likely to occur within PSII, and should prove useful in the design of artificial catalysts for water oxidation.

$$\text{(dry S0) Mn}^{II} + H_2O \longrightarrow \text{Mn}^{II}\text{-OH}_2 \qquad \text{-4 kcal/mol}$$

$$\text{Mn}^{II}\text{-OH}_2 + Y_Z^{\bullet} \longrightarrow \text{(S1) Mn}^{III}\text{-OH} + HY_Z \quad \text{-7 kcal/mol}$$

$$HY_Z + OH^{\bullet} \longrightarrow Y_Z^{\bullet} + H_2O \qquad \text{-32.5 kcal/mol}$$

$$\text{Mn}^{II} + OH^{\bullet} \longrightarrow \text{Mn}^{III}\text{-OH} \qquad \text{-43.5 kcal/mol}$$

A comparison of Photosystem II with other metalloradical enzymes

In 1995, strong similarities between Photosystem II and the class of metalloradical enzymes were becoming apparent, and this trend has continued to the present. The rebound mechanism of cytochrome P450 has already been mentioned as an example of a radical trapping reaction. Galactose oxidase exhibits mechanistic cooperation between a radical and a transition metal atom similar to that proposed for the O-O bond forming reaction in PSII. The greatest mechanistic similarities, though, are those emerging between PSII and cytochrome *c* oxidase.

Cytochrome oxidase catalyzes the reduction of oxygen by cytochrome *c* with water as product, so the reaction is very nearly the reverse of the PSII reaction. Both enzymes are capable of hundreds of turnovers per second. The active site for oxygen reduction contains a heme and a copper atom ligated by three histidine residues, one of which is crosslinked to a tyrosine residue, Y244. The crosslink, reminiscent of the cysteinyltyrosine crosslink in galactose oxidase, is a strong indicator of radical reactions occurring within the active site. Based on these data, we predicted that Y244 would donate a hydrogen atom during the cleavage of the O-O bond (Hoganson et al., 1998).

Recent work from the Babcock lab (Proshlyakov et al., 2000) has confirmed that the Y244 is redox-active and that it donates a hydrogen atom during reduction of O_2. Density functional modeling of the active site reactions (Blomberg et al., 2000) has located two transition states in which hydrogen atom donation by the tyrosine occurs concertedly with breaking the O-O single bond, as in the predicted mechanism. The ferryl species formed at the heme is estimated to have an $Fe^{IV}=O$ bond strength of 58 kcal/mol, in line with the $Mn^{IV}=O$ bond strength of 62 kcal/mol we estimated for S_3 of PSII. The cytochrome oxidase transition structures found by modeling require, for geometric reasons, the participation of an additional water molecule. The estimated activation energies are rather high, however, perhaps due to technical limitations of the calculations. Nevertheless, I think the modeling results lend additional credence to the mechanisms we have proposed for Photosystem II and cytochrome *c* oxidase.

Future Prospects

The metalloradical mechanism of photosynthetic water oxidation has been illustrated in previous publications as simply as was thought possible. Additional data such as the protein

structure from crystallography (Zuounl et al., 2001) are likely to warrant refinements to the model. In addition to the ligands to manganese and their arrangement, the location of additional water molecules and hydrophilic amino acid residues are likely to be revealed. No doubt these will help explain some of the more intriguing observations connected with Photosystem II. It nonetheless appears that the broad outlines of the water oxidizing mechanism, the physical chemical principles that govern the reaction, have become clear. It is my hope that knowledge of these principles will inspire and guide the synthetic chemists among us to design and construct new catalysts for water oxidation and oxygen reduction. Such catalysts will surely prove useful in fuel cells and for solar energy conversion.

Acknowledgments

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