From water to oxygen and back again: mechanistic similarities in the enzymatic redox conversions between water and dioxygen

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Abstract

We propose that the interconversions of water and oxygen are catalyzed by the transition metal ions of Photosystem II and cytochrome c oxidase in remarkably similar ways. Oxygen–oxygen bond formation and cleavage occurs between two oxygen atoms that are bound as terminal ligands to two redox-active metal ions. Hydrogen atom transfer to or from a tyrosine residue is an essential component of the processes in both enzymes. © 1998 Elsevier Science B.V.

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1. Introduction: importance

Aerobic life owes its existence to Photosystem II and its diversity to cytochrome c oxidase. These two enzymes are responsible, respectively, for essentially all of the creation of dioxygen and for most of its consumption by living organisms.

The presence of an oxygen-rich atmosphere was made possible by the development of Photosystem II, a protein–pigment–cofactor complex that is found in cyanobacteria, algae and higher plants [1–5]. It uses the energy of light to drive the oxidation of water to dioxygen, a redox process involving four electrons for every molecule of product O₂ released. The development of an oxygen-rich atmosphere gave impetus to the evolution of cytochrome oxidase, a protein–cofactor complex that is found in all aerobic organisms [6–8]. It uses O₂ as the molecular oxidant in respiration, thereby enabling a much more exergonic metabolism of organic substances than is possible otherwise, as in fermentation, for example.

In the enzymatic process catalyzed by cytochrome c oxidase, the oxygen atoms of O₂ are released as product water.

Clearly, these two enzymes form an important portion of the biogeochemical oxygen cycle. Interest in these enzymes is high because of their unique importance and because of the insights into catalysis that may become available from a full understanding of their catalytic cycles. Research into their mechanisms has continued to progress and a number of developments have made it possible now to see some features that may be important for both enzymes. In particular in this article, we will focus on the sites of oxygen bond formation and cleavage and on the...
details of the molecular processes involved in these two critical reactions.

2. Reactions: co-reactants, protons, and energetics

2.1. Photosystem II

The chemically balanced reaction catalyzed by PSII shows the reduction of two plastoquinone molecules per O₂ produced. In addition, four protons are released to one side and taken up from the other side of the thylakoid membrane. The proton gradient created is used for ATP synthesis. The reducing equivalents represented by the plastoquinol product are eventually used in the reduction of CO₂ and NO₃.

To carry out this chemistry, the photochemical reaction center of PSII contains six chlorophyll molecules, two pheophytin molecules a non-heme iron atom, a tightly bound plastoquinone, and the binding site for an exchangeable quinone [1]. These cofactors are bound to a heterodimeric integral membrane protein whose structure is likely to be rather similar to that of the non-oxygen-evolving photoreaction centers from purple bacteria whose structures have been determined by X-ray crystallography. Although, no high-resolution structure is yet available for PSII, the sequences of the two integral polypeptides, D1 and D2, are available for many species and form the basis for building detailed structural models.

The reaction center chlorophyll and pheophytin receive excitation energy and transfer one electron per photon to the bound quinones. This process generates a chlorophyll cation radical P₆₈₀⁺, which is a strong oxidant, and which is reduced by electron transfer from tyrosine Y₂, Y161 of the D1 polypeptide to generate a tyrosyl radical. The EPR spectrum of Y₂ can be measured and has been studied extensively. This tyrosyl radical, in turn, oxidizes a cluster of four manganese atoms at which water binds and from which O₂ is released. The manganese cluster [2,3] is the accumulator of oxidizing equivalents and goes through a cycle of oxidation states, S₀ to S₄ (Fig. 1a). S₄ is the most oxidized and decomposes spontaneously releasing O₂. One calcium ion and one chloride ion are also required for full activity.

Information from XAFS has been used to develop the structural model of the manganese cluster [3] that has lately received the most attention. It contains two magnetically interacting di-μ-oxo-bridged manganese dimers, in each of which the Mn–Mn distance is 2.7 Å. In the favored model, the two Mn₃O₂ units are connected by a further bridging oxo ligand so that the Mn₄O₅ cluster takes the shape of a C, with Mn–Mn distances of 3.3 Å at the closed end and 5.5 Å at the open end. EPR measurements on inhibited samples suggest a distance between the manganese cluster and the tyrosyl radical of about 5–10 Å.

![Diagram](image-url)

Fig. 1. The catalytic cycles of Photosystem II and cytochrome c oxidase. For Photosystem II, only the redox-active, substrate-binding manganese ions are shown. For cytochrome oxidase, only the binuclear center is shown.
2.2. Cytochrome oxidase

The immediate source of reducing equivalents for the conversion of O\textsubscript{2} to water is cytochrome c; ultimately, the source is the oxidative catabolism of organic compounds. Protons are involved in the reaction of cytochrome oxidase, with eight taken up from one side and four released to the opposite side of the inner mitochondrial membrane. As above, the proton gradient that results from this vectorial chemistry is used to drive the synthesis of ATP. Four of the protons involved are used to reduce O\textsubscript{2} and are referred to as ‘chemical’ or ‘scalar’ protons. The other four are transported across the membrane and are referred to as ‘pumped’ or ‘vectorial’ protons.

Cytochrome oxidase [6-8] is a multisubunit integral membrane protein, and X-ray-determined structures are available for the protein from two species [9-12]. Subunit I binds two heme a molecules and a copper ion. Subunit II binds a dimeric copper cluster, Cu\textsubscript{A}. Oxygen reduction occurs in subunit I at the binuclear site composed of one heme a (historically called heme a\textsubscript{3}) and the copper ion (called Cu\textsubscript{B}). Cu\textsubscript{B} and the iron atom of heme a\textsubscript{3} are 4.5-5.2 Å apart [9-12]. Electrons from cytochrome c are passed first to Cu\textsubscript{A}, then to heme a, then to heme a\textsubscript{3}. An interesting feature of the binuclear site is that one of the ligands to Cu\textsubscript{B} is a histidine that has been covalently crosslinked to a tyrosine residue (Yoshikawa, S., personal communication; [12]). The tyrosine phenolic oxygen atom is about 5 Å from the heme a\textsubscript{3} Fe, and its bound proton is ideally positioned to hydrogen bond to substrate O\textsubscript{2} bound to a\textsubscript{3}.

The binuclear site can exist in different oxidation states (Fig. 1b). The reduced state, Fe\textsuperscript{2+}Cu\textsuperscript{1+}, exists in the absence of O\textsubscript{2}. When O\textsubscript{2} binds, the oxy state (O) is formed. The O state reacts within microseconds to give the P state, which has often been formulated as Fe\textsuperscript{3+-OO}Cu\textsuperscript{2+} [13], but this formulation is disputed (see below). Electron transfer from heme a to the binuclear site then gives state F, in which the O-O bond has been indisputably cleaved, and a ferryl iron (Fe\textsuperscript{4+-O}) is detected by Raman spectroscopy [7,14]. A second electron into the site gives the oxidized state, Fe\textsuperscript{3+}Cu\textsuperscript{2+}. Two more electrons regenerate the reduced state of the binuclear center.

3. O-O bond formation in PSII

We recently proposed a detailed mechanism for O-O bond formation in PSII [15]. This proposal builds upon our ideas about the function of the tyrosyl radical as a hydrogen atom abstractor from substrate water bound terminally to the two Mn ions at the open end of the C-shaped (Mn\textsubscript{4}) cluster. This role for Y\textsubscript{Z} is possible because of its close proximity to the manganese cluster and is supported by the general action of tyrosyl radicals in enzymatic catalysis as hydrogen atom abstractors [16,17]. The thermodynamic feasibility of this proposal has been strongly supported by the experimental and theoretical determinations of the O-H bond strengths of water and hydroxide bound to manganese ions [18-21]. Other features of this model are that the manganese cluster remains essentially electrically neutral, and that oxygen is formed from terminal ligands to the cluster. The model has now received a good deal of further experimental support, including the exchange rates of bound substrate, the kinetics of proton release, deuterium kinetic isotope effects, and mutational analysis, and is consistent with the theoretical analyses of the thermodynamics and kinetics associated with the water oxidation reaction [15,17,22].

The recent proposal for O-O bond formation during O\textsubscript{2} evolution in PSII views the S\textsubscript{3}Y\textsubscript{Z} reaction again as having a hydrogen atom transfer component [15]. The S\textsubscript{3} state, however, is composed solely of Mn\textsuperscript{4+} ions, and advance to the next higher oxidation state, analogous to that which occurs in the lower S state transitions, is more difficult. S\textsubscript{3} is proposed to contain one Mn\textsuperscript{4+-OH} and one Mn\textsuperscript{4+-O} at the open end of the cluster. O-O bond formation is seen as a concerted reaction in which the tyrosyl radical abstracts the hydrogen atom, and the remaining oxygen adds to the oxygen of the Mn\textsuperscript{4+-O}, producing a single O-O bond and Mn\textsuperscript{3+} ion. The peroxo species that results from this concerted reaction decomposes immediately to release O\textsubscript{2}; it is not clear whether the peroxo species is even a temporarily stable species. This proposal explains the surprising fact that reduction of the tyrosine and release of O\textsubscript{2} occur with essentially the same kinetics [23].

The proximity of the two oxygen atoms makes this reaction possible and also makes its kinetics sensitive.
to structural perturbations around the manganese cluster. Depletion of neighboring water-soluble polypeptides or replacement of the active site calcium ion with strontium both slow Y\textsubscript{2} reduction and O\textsubscript{2} release substantially (e.g., [24]).

4. Oxygen–oxygen bond cleavage in cytochrome c oxidase

As indicated above, the O–O bond is cleaved between the oxy and the ferryl intermediates [13]. Between those intermediate states, lies the P state, and a key question for recent work has been to discover whether the O–O bond is intact or cleaved in this species. The P state is distinguished from both O and F by its absorbance and Raman spectra. The most natural way to produce the P state is by reaction of the two-electron-reduced enzyme with O\textsubscript{2}, but it can also be obtained by reaction of the oxidized enzyme with H\textsubscript{2}O\textsubscript{2} or by a reversal of the usual reactions driven by a large pH gradient across the membrane [7,8,25,26]. Raman spectra of the state produced in the reaction with H\textsubscript{2}O\textsubscript{2} suggest that the P state already contains a ferryl moiety, implying that the O–O bond has been cleaved. Recent work in our lab [26] shows the same result for the reaction of O\textsubscript{2} with the two-electron-reduced enzyme, namely that the P state contains ferryl heme a\textsubscript{3} (Fe\textsuperscript{4+}=O).

The P state therefore occurs after the four-electron reduction of dioxygen to produce oxo and hydroxo species. Heme a\textsubscript{4} provides two electrons (Fe\textsuperscript{2+}→Fe\textsuperscript{4+}) and Cu\textsubscript{b} (1+→2+) provides a third electron. The source of the fourth electron has been unclear until recently, but crystallographic data (Yoshikawa, S., personal communication; [12]) now strongly suggest that the crosslinked tyrosine is redox-active and can donate an electron, or rather a hydrogen atom during the reduction of O\textsubscript{3} [26]. The bond cleavage reaction can then be formulated as a rapid, concerted reaction of the true, but undetectable, bridging peroxy intermediate and the tyrosine. As the tyrosine donates a hydrogen atom to the oxygen bound at Cu\textsubscript{b}, the O–O bond cleaves and the iron is oxidized to the Fe\textsuperscript{4+}=O ferryl form. Cu\textsubscript{b} remains in the 2+ state during this process. The first electron into the site from heme a returns the tyrosyl radical to the diamagnetic tyrosine of state F.

5. Common features of H\textsubscript{2}O/O\textsubscript{2} reactivity in photosynthesis and respiration

Cytochrome oxidase and Photosystem II both have rapid rates of turnover, maximally several hundred electrons per second, consistent with their importance in the energetic economies of their host cells. In both systems, the singly bonded peroxy intermediates have, so far, avoided spectroscopic detection, because of their short lifetimes. This is consistent with our expectations about the energetic properties of species containing weak O–O bonds. Nevertheless, because it is logical that a singly bonded species should be an intermediate between those containing either zero or two bonds, we describe the reactions in terms of these intermediates.

The oxygen–oxygen bond formation and cleavage reactions in PSII and cytochrome oxidase, respectively, are catalyzed in strikingly similar fashions (Fig. 2). The geometrical arrangement of the two transition metals and the redox-active tyrosine are similar. The concerted reactions involve a chain of five atoms: O\textsubscript{tyr}–H–O–O–Mn/Fe, and bond order is conserved in the transformation. The reactions have an essentially radical nature, and involve hydrogen atom transfer between the substrate and the phenolic oxygen of a tyrosine residue. The oxidation state of only one metal ion of the several present in the active sites is changed in this crucial step, and only one of the bonds formed or cleaved, the metal–oxygen bond,
has significant ionic character, the rest being essentially covalent.

The reactions differ in their directionality and in the actual cofactors utilized. PSII utilizes manganese and an unmodified tyrosine because these can be made to have reduction potentials intermediate between those of $P_{680}^+$ and the $O_2/H_2O$ couple. For rapid reduction of $O_2$ by cytochrome c, it is essential that at least the majority of the cofactors have reduction potentials between those of $O_2/H_2O$ and the cytochrome, which heme $a_3$ and $Cu_B$ do. The effect of the cross-link to the tyrosine in cytochrome oxidase is probably to make it a better reductant, particularly when $Cu_B$ is oxidized.

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References