

# Elements of Life at the Oxo Wall

by Harry B. Gray

**W**e live on a planet bathed in dioxygen. Iron, the most abundant transition metal element on Earth, reacts with dioxygen to form rust and red clays. It is also an essential component of enzymes that generate the energy we need to live, but alas, many of these reactions can be harmful, leading eventually to destruction of vital organs in our bodies. We can't live without iron and dioxygen, but there are challenges living with them!

## Introduction

The top two chemical reactions on Planet Earth are water splitting to dioxygen in photosynthesis and dioxygen reduction in mitochondria during respiration. The elements in the protein machines that drive these reactions are manganese and iron. Both of these elements function in complexes in which the metal center is bonded to an oxygen atom, or more correctly, an oxide anion, in a molecular unit called a "metal-oxo." A manganese-oxo cluster is required for water oxidation in green leaves, producing dioxygen, and an iron-oxo is the active intermediate in dioxygen reduction to water in cytochrome oxidase, the terminal enzyme in respiration [1].

We could not live without proteins that contain iron. Arguably the most important iron protein is cytochrome oxidase, but hemoglobin, which carries dioxygen in our blood, is a close second. In a PNAS Perspective, Kara Bren, Rich Eisenberg, and I retold the 75-year-old (and often very controversial) story of iron-dioxygen bonding in heme proteins [2]. Among the many other iron proteins, myoglobin, which stores dioxygen in our bodies, the cytochrome electron transferases, and the oxidative iron enzymes, deserve special mention.

Of interest in this essay is the oxidative enzyme cytochrome P450, or just "P450." P450 catalyzes the oxidation of a vast array of biologically important organic molecules. When dioxygen reacts with the iron in P450, the powerfully oxidizing multiply bonded iron-oxo formed can break a very strong carbon-hydrogen bond in a hydrophobic molecule, turning it into a water soluble alcohol. Syntheses of many bioactive molecules depend critically on P450 catalyzed oxidations. When the oxidized P450 snatches an electron from the C-H bond, the oxo attracts the C-H proton, forming a protonated oxo and a "carbon radical." The protonated oxo then quickly binds to the carbon radical in a reaction called the "rebound," producing a water soluble alcohol [1].

## Ligand Field Theory

How do manganese and iron bond to oxos? And how do these metal oxos function in biological reactions? The story starts early in 1961, when I was working with Carl Ballhausen, a professor at the University of Copenhagen. I had shown in the fall of 1960 that the crystal field theory could not account for the energies of absorptions in the near infrared and visible spectrum of the nickel aquo ion. As it was clear to me that a better theoretical framework was needed, I decided to modify Robert Mulliken's molecular orbital (MO) theory, which was good for organic molecules but had given crazy results for inorganic ones. I invented a way to correct the diagonal energy elements so MO calculations gave one-electron orbital energies in accord with experiment. Then I picked an oxo of vanadium, the vanadyl ion, to test this "Ligand Field Theory." My calculations were successful, accounting not only for the vanadyl absorption spectrum, but also for its magnetic and reactivity properties. I wrote two papers on ligand field theory, one with Ballhausen, another with Curt Hare, in which I showed that oxos form triple bonds to vanadium, chromium, and molybdenum [3,4]. And I predicted that protons would not be attracted to triply bonded oxos, rather that nucleophilic reagents likely would react with them. It is of interest in this context that one of the proposed mechanisms of dioxygen evolution during photosynthetic water oxidation features hydroxide attack on a triply bonded Mn-oxo [5]. But there are other proposed mechanisms, some with substantial support from both theory and experiment, so the dust has not settled yet on this important area of science.

## The Oxo Wall

In teaching inorganic chemistry at Caltech, I always have emphasized that early transition metal elements react with dioxygen to form multiply bonded metal-oxos. I have discussed the bonding in metal-oxos every year, with emphasis mainly on the vanadyl ion. One year I decided to add tetrahedral oxos such as chromate and permanganate to the story. In preparing for class, I remembered from searches I had done in Copenhagen that there were no stable tetraoxos of transition metal elements beyond the iron-ruthenium-osmium column in the periodic table. Nor were there any tetragonal metal-oxos past that column. I consulted my tetrahedral and tetragonal ligand field energy diagrams, discovering that I had predicted that strongly bonded metal-oxos could not exist if there were more than two  $\pi$ -antibonding electrons. On that very day I told the class that there must be an "oxo wall" between

the Fe-Ru-Os and Co-Rh-Ir triads in the periodic table (Figure 1)!

My oxo wall lecture was a highlight every year in the course. I decided to take it public, giving “oxo” seminars at universities and at ACS meetings. For years the only “oxo wall” publications were ACS abstracts (Jay Winkler and I have corrected that oversight [6,7]). But these were enough to energize inorganic chemists to search for violations. The search came up empty until a well-known investigator reported in *Science* that he had made a tetragonal Pt-oxo compound. I knew that a Pt-oxo would be a violation! I was relieved when he sent me an email saying that he was retracting his claim, as upon further study, it turned out that his platinum-oxo was in fact a tungsten-oxo, which was on the left (allowed!) side of the wall. He did me a great favor when, in the title of his “tungsten-oxo” paper, he wrote, “The Oxo Wall Stands” [8].

Nature knew about the oxo wall when she picked elemental metals for the generation and reduction of dioxygen. In their reaction cycles, manganese in photosystem II and iron in cytochrome c oxidase and P450 form multiply bonded metal-oxos with dramatically different properties (Figure 2). Metal-oxo electronic structure is key: a protonated triply bonded Mn(V)-oxo is a super acid, with a  $pK_a$  less than -10. Such a highly acidic oxo could attract an oxygen donor, which in turn would promote redox-coupled O-O bond formation. In contrast, the combination of two  $\pi^*$  electrons and

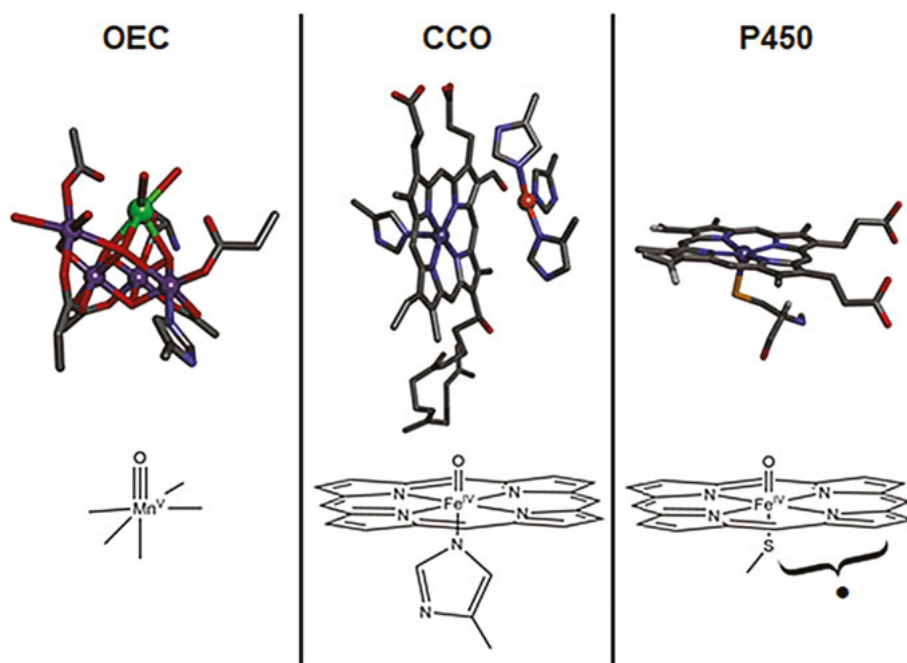
an axial cysteine thiolate combine to make the doubly bonded Fe(IV)-oxo of P450 compound II very basic, with a  $pK_a$  of about 12. It is widely recognized that the generation of such a basic Fe(IV)-oxo is an essential step in the catalytic cycle of P450 [7,9].

### Cytochrome P450: Good or Bad?

Mike Green, a professor at UC Irvine, has pointed out that compound I of P450 would be able to extract an electron from a C-H bond only if coupled to proton transfer to a newly formed Fe(IV) oxo of compound II. He has shown that the high reduction potential of compound I and the basicity of compound II together provide the driving force for the reaction [9,10]. In this reaction, the newly formed hydroxyl and carbon-centered radicals rebound, making a C-O bond in forming

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| Na | Mg |    |    |    |    |    |    |                                                                                     |    |    |    | Al | Si | P  | S  | Cl | Ar |    |
| K  | Ca | Sc | Ti | V  | Cr | Mn | Fe |  | Co | Ni | Cu | Zn | Ga | Ge | As | Se | Br | Kr |
| Rb | Sr | Y  | Zr | Nb | Mo | Tc | Ru |                                                                                     | Rh | Pd | Ag | Cd | In | Sn | Sb | Te | I  | Xe |
| Cs | Ba | Lu | Hf | Ta | W  | Re | Os |                                                                                     | Ir | Pt | Au | Hg | Tl | Pb | Bi | Po | At | Rn |

**Figure 1.** There is an oxo wall between Fe-Ru-Os and Co-Rh-Ir in the periodic table. There are no multiply bonded (tetragonal) metal-oxos with more than 4 d electrons, in accord with the predictions of ligand field theory. Multiply bonded metal-oxos beyond the wall cannot exist, as the metals in the high oxidation states required [for example, Co(V) or Ni(VI)] would be rapidly reduced in an oxo ligand environment.



**Figure 2.** Manganese oxos in the oxygen evolving complex (OEC) of photosystem II catalyze water oxidation to dioxygen; an axial histidine-ligated iron-oxo in cytochrome c oxidase (CCO) is a key intermediate in dioxygen reduction during respiration; and an axial cysteine-ligated P450 compound I iron-oxo converts hydrocarbons to molecules that are critical for life.

the product alcohol, along with return of the heme iron to its resting (ferric) state. The million dollar question then becomes, what happens if the substrate is not oxygenated? If the powerfully oxidizing compounds I and II are generated in the absence of substrate, what keeps them from destroying the proteins (and the cells) they occupy [11]?

In the P450 reaction cycle, the heme iron in the resting enzyme is in the 3+ oxidation state. Substrate binding in the heme pocket displaces an axial water ligand, resulting in a positive shift in the  $\text{Fe}^{\text{III/II}}$  formal potential. This increased oxidizing power favors electron delivery from cytochrome P450 reductase to produce the  $\text{Fe}^{\text{II}}$  state, which is followed by dioxygen binding to produce an  $\text{Fe}^{\text{III}}$  superoxide complex. Delivery of a second electron from the reductase induces O-O bond scission, forming water and the hydroxylating agent, iron-oxo compound I. Hydrogen atom abstraction from the substrate generates iron-oxo compound II and a substrate radical; hydroxyl rebound produces the product and water ligation regenerates the enzyme resting state [1].

During cytochrome P450 catalysis, one molecule of  $\text{O}_2$  is consumed for each molecule of product formed. The human liver enzyme known as CYP3A4 metabolizes many therapeutic drugs; and it couples  $\text{O}_2$  consumption to substrate oxidation, but with only 10% efficiency [12]. In 2014, Jay Winkler and I proposed that redox chains comprised of tyrosine and tryptophan residues (Tyr/Trp chains) could protect the enzyme from

damage by compound I when reaction with substrate does not occur [13]. We proposed that these Tyr/Trp redox chains could guide oxidizing equivalents (called “holes”) away from the critical active site, taking them to the enzyme surface where they could be scavenged by soluble reductants such as glutathione and ferrocytochrome  $b_5$ . In search of support for our proposal, we examined thousands of X-ray crystal structures in the RCSB Protein Data Bank (PDB), finding that Tyr/Trp chains occur prominently in oxidoreductases [14]. Based on this finding, we suggested that there is an internal antioxidant protection mechanism involving long-range electron transfer from Tyr (or Trp) to the heme iron, where the internal antioxidant time constant is determined by the distance from the heme to the nearest Tyr/Trp residue. If the P450 iron-oxo fails to oxidize substrate within the prescribed time limit, electron transfer from the Tyr/Trp chain would rescue the enzyme by regenerating the ferric resting state.

We found that Tyr/Trp-to-heme distances in P450s are often in the 7–8 Å range. Of interest is that many structurally characterized eukaryotic P450s are mammalian enzymes where, in each case, a tryptophan is hydrogen bonded to a heme-propionate. The tryptophan in question in human CYP3A4 is Trp126, located 7.2 Å from the heme and 4.7 Å from a surface exposed tyrosine, Tyr99. In this enzyme, there is a potential protection pathway involving hole transfer from the heme to Trp126, then moving on to Tyr99. Based on multistep electron tunneling times from previously constructed “hopping



maps," we estimated that the iron-oxo of CYP3A4 compound I would be reduced by Trp126 in 200 ns if substrate oxidation did not occur more rapidly [7]. In this scenario, the Tyr99 radical generated by hole hopping from Trp126 would react with soluble reductants such as glutathione. Trp 126 sends a very clear message to the heme: "Look, if your iron-oxo doesn't oxidize the substrate, I am going to reduce you!"



### Parting Shots

We have been living with dioxygen for over two billion years. Manganese plays a key role in generating dioxygen, and iron enzymes use it to make compounds critical for human life. But dioxygen is toxic, and Nature has had to evolve protection mechanisms to keep us alive! 🦎

### Acknowledgment

My research in biological inorganic chemistry has been supported for many years by the United States National Institutes of Health, the National Science Foundation, and the Arnold and Mabel Beckman Foundation.

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Harry B. Gray <hbgray@caltech.edu> is the Arnold O. Beckman Professor of Chemistry at California Institute of Technology, in Pasadena, California.

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