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Biochemistry. An ancient and intimate partnership

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Prior to the evolution of photosynthesis ~ 3.5 billion years ago, Earth's atmosphere was reducing, and iron was predominantly in its Fe(II) oxidation state. The abundance of Fe(II) soaked up the early oxygen generated by photosynthesis in a 1.5 billion year long rusting event. The evolution of complex multicellular organisms had to wait until substantial amounts of oxygen became available in Earth's atmosphere ~ 2 billion years ago. Then the tables were turned, and organisms used the abundant iron to access the oxidative power of oxygen, and thus dramatically increase the efficiency of metabolic energy generation. In this issue the biological intimacy of this relationship is highlighted in two articles that focus on mononuclear non-heme iron enzyme systems. The first by Kovaleva and Lipscomb (1) reveals the details of how a protein can subtly tune an Fe(II) ion to activate kinetically sluggish O₂ to enable the oxidation of aromatic diols in a 4 electron metabolic process. In contrast, the Katona *et al.* study (2) highlights the "dark side" of the metabolic coin, where toxic byproducts of oxygen reduction, known as reactive oxygen species (ROS), need to be rapidly removed from living organisms to prevent cellular damage.

The mononuclear non-heme Fe(II) enzymes that contain a 2-His-1-carboxylate facial triad are a large and diverse group (3). This Fe(II) ligating motif has evolved multiple times in different protein scaffolds, and the enzymes catalyze crucial chemistries in the synthesis of neurotransmitters, antibiotics and plant hormones. The bacterial extradiol ring-cleaving dioxygenase studied by Kovaleva and Lipscomb is a key enzyme in the metabolic degradation of aromatic hydrocarbons introduced into soil by man. These researchers used a slow substrate and a low O₂ atmosphere to freeze trap three different catalytic intermediates in a crystal of the enzyme. Two of these capture the intimate partnership between iron, dioxygen and 1,2-dihydroxylated aromatic substrate. The iron orchestrates the dance between dioxygen and substrate, acting as a conduit to enable electron density to move from substrate to molecular oxygen. This alters their character and creates two reactive species; a superoxo and a substrate radical. The superoxo species is unexpectedly side-on to the iron in a weak interaction due in part to steric crowding from the five other ligands to the metal (figure, left panel). This perfectly aligns the superoxo to attack the substrate radical and form the alkyl-peroxo intermediate, also visualized for the first time in this study (figure, middle panel).

The study by Katona *et al.* concerns an enzyme involved in superoxide anion radical O₂^{•-} detoxification, and is found in a range of bacteria and archaea (4). Superoxide reductase converts superoxide to hydrogen peroxide (H₂O₂) in a one electron reduction. The role reactive oxygen species, such as O₂^{•-}, play in human pathology and ageing has led to an upsurge in interest in how damaging radicals are generated and detoxified within biological systems. These researchers have freeze trapped a number of (hydro)peroxide configurations with Fe(III) within a crystal of an enzyme mutant that stabilizes the Fe(III)-peroxo. In contrast to the other study, these are end-on complexes (figure, right panel). The different oxidation states of dioxygen (molecular oxygen, superoxide, peroxide) focus attention on the electron transfer events, but equally important are the source and transfer of two protons during the reduction from O₂^{•-} to H₂O₂. The source of protons can be amino acids,

cofactors or water, and enzymes exquisitely control the protonation steps in perfect synchrony with electron transfer. The active site structures presented here, although all ostensibly of the same Fe(III)-peroxo species, display three different end-on peroxide conformations through rotation on the Fe(III) pivot, thus linking the peroxo to waters and amino acids within the active site. The concerted movements of these players suggest proton transfer pathways, and this study represents the most complete insight into protonation through rotation to date. The identity of peroxide is confirmed by the presence of ^{18}O isotope sensitive bands within the correct vibrational range for Fe(III)-peroxo complexes assessed by Raman spectroscopy in solution and crucially also in the crystal. Density Functional Theory calculations performed on the structures suggest that at least two are hydroperoxides.

The role of crystal packing in trapping these species becomes apparent when you realize that the data used to generate all the intermediates came from a single crystal in each study. In each case there were four independent copies of the active site in the basic crystal building block, enabling four different images of the active site to be observed simultaneously. The different species in each study manifested themselves in structurally distinct active sites. The reason why different crystal packing environments stabilized different species is beyond the resolution of these data, and suggests that subtle structural effects within the protein remain key to controlling the chemistry. As demonstrated in the superoxide reductase study, the combination of higher resolution spectroscopic experiments, both in the crystal and in solution, and the use of computation methods coupled with the crystal structures, represents a potent toolbox that promises in the future to unlock many secrets within chemical biology.

Together these two studies represent a stunning insight into the diverse structural relationships mononuclear non-heme iron enzymes can broker between oxygen, protein, solvent and hydrocarbon substrates. The iron centers in these enzymes retain great flexibility in their co-ordination spheres compared to their heme cousins, who are constrained by their porphyrin partners. This fact probably explains the great diversity of substrate chemistries that can be catalyzed by these enzymes.

If the Earth did not have such an abundant supply of iron, by this point in geologic time we could be a billion years more evolved. In that scenario, iron and oxygen would probably not be so intimately linked within the fabric of biological life, leading to loss of chemical richness associated with this versatile transition metal. That in my mind would represent a much less interesting state of affairs.

Acknowledgments

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References

1. Kovaleva EG, Lipscomb JD. *Science*. 2007
2. Katona G, et al. *Science*. 2007
3. Koehn top KD, Emerson JP, Que L Jr. *J Biol Inorg Chem*. 2005; 10:87. [PubMed: 15739104]
4. Niviere V, Fontecave M. *J Biol Inorg Chem*. 2004; 9:119. [PubMed: 14722742]

Figure. Iron-dioxygen relationships

The plasticity of the non-heme iron environment enables the accommodation of a diverse range of geometries with substrates and dioxygen, as illustrated here by trapped catalytic intermediates in an extradiol ring-cleaving dioxygenase (**left and middle panels**) and superoxide reductase (**right panel**).