The shortest wire

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Catechol dioxygenases catalyze the opening of an aromatic ring and incorporation of both atoms of a molecule of dioxygen (reviewed by ref. 1; see Scheme 1). Initial deprotonation of a ring hydroxyl facilitates oxidation to a semiquinone-type state and loss of aromatic stabilization of the ring structure, but O_2 also requires activation for the reaction to occur. Although the overall reaction is thermodynamically favorable, it is kinetically impeded by O_2's spin triplet nature in the ground state, because this spin is not conserved in the reaction's singlet products (2). Thus, the catalyzed reaction must at least include mechanisms for recombination of electron spins, deprotonation of catechol, electron transfer from catecholate and to O_2, and the chemical steps that follow. The early functions and proximate binding of O_2 and catecholate can be performed by appropriate redox-active metal ions, so it is natural to focus on the metal ion when one is present. However, each of the early requirements can also be met by other means. In this issue of PNAS, Emerson *et al*. (3) use a pair of catechol dioxygenase enzymes to remind us of the versatility and variety of biochemical mechanisms. They show that despite the compelling elegance and many capabilities of redox-active metal ions as hammers, not all reactions are nails. Specifically, they argue that the Mn^{2+} of homoprotocatechuate 2,3-dioxygenase from *Arthrobacter globiformis* (MndD) and the Fe^{2+} of homoprotocatechuate 2,3-dioxygenase from *Brevibacterium fuscum* (Fe-HPCD) mediate reduction of O_2 by bound catecholate without themselves undergoing formal oxidation.

The Fe^{2+}-dependent catechol dioxygenases (CdOs) have Fe-binding sites that fall within a large and diverse host of so-called "two histidine, one carboxylate facial triad" (2H1E) enzymes in which Fe^{2+} is bound by one Glu/Asp and two His side chains on adjacent apices of the coordination octahedron, leaving three labile coordination sites for bidentate binding of a catecholate adjacent to dioxygen (4). This arrangement is very attractive from a chemical standpoint because it positions dioxygen close to both of the catecholate positions that are activated by binding to Fe (Fig. 1). The protein structural context of the active site varies, and it appears that 2H1E-triad sites have evolved on multiple independent occasions by convergent evolution (1).

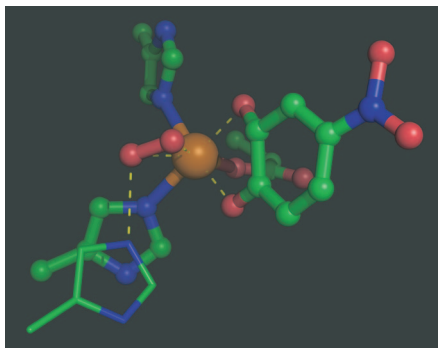

Fig. 1. X-ray crystal structure of ternary complex between Fe-HPCD, O_2, and 4-nitrocatechol, based on the structure of Kovaleva *et al*. (7), PDB ID code 2IGA chain C. O_2 appears bound in a side-on conformation with an H bond from the conserved His200. 4-Nitrocatechol coordinates *trans* to the two His ligands. Also see ref. 11. This figure was generated by using PyMOL (12).

Catechol dioxygenation is thermodynamically favorable, and the catalyst's job may be simply to free the cork from the bottle. Once the reactive driving force of O_2 is loosed, all manner of chemistry can follow spontaneously. The cork is the kinetic barrier posed by O_2's spin triplet nature. Many enzymes overcome the spin barrier by reducing O_2 to superoxide, whose single unpaired spin can combine with another unpaired spin or that of the metal ion (5, 6).

Fe-HPCD displays a superoxo intermediate, but in this case its formation is clearly accompanied by oxidation of the catecholate (7), arguing against oxidation of the metal ion. This has moreover been supported computationally (8). The Emerson article provides additional proof that redox activity on the part of the Fe is not required, by replacing Fe with Mn.

The teams of Lipscomb, Que, and Wackett over the years have built a body of evidence supporting the strong analogy between the Fe^{2+}-containing CdO Fe-HPCD and a rogue CdO that employs Mn^{2+} instead. The two share strong structural homology (9) and very similar catalytic constants [see the work of Emerson *et al*. (3)]. So much has also been shown for the native Fe-SOD and Mn-SOD of *Escherichia coli* (reviewed in ref. 10). However, that is where the similarity ends. The two superoxide dismutases (SODs) apply very different redox tuning to the bound metal ion, thus compensating for the many hundreds of mV difference between the intrinsic reduction midpoint potential of $Mn^{3+/2+}$ (adding one electron to an empty d-orbital) vs. that of high-spin $Fe^{3+/2+}$ (adding an electron to an occupied d-orbital). Thus, the SOD proteins, with their greater number of protein ligands and their crucial coordinated solvent molecule that couples proton transfer to the redox event, achieve/very similar reduction midpoint potentials in FeSOD and MnSOD. However, Fe-substituted-MnSOD and Mn-substituted-FeSOD are virtually inactive under standard conditions.

In the CdOs described by Emerson *et al.* (3), any differences in the redox tuning applied by the Fe-HPCD and MndT proteins does not appear to be crucial to activity, because both proteins achieve similar activity with either metal ion. The bound metal ions appear to retain much of their different redox characters, in each of the two protein contexts, as the authors find that the Fe is more prone to oxidation (lower E_m) than the Mn (although the signature of some differential tuning is revealed by Fe-MndD's greater susceptibility than Fe-HPCD's). This is not unexpected. However, what is new is that even when Fe^{2+} is bound in the protein evolved to use Mn^{2+}, it retains full activity, and similarly for Mn^{2+} bound to HPCD protein. Thus, even when any evolved protein-derived redox tuning should in fact reinforce intrinsic differences between Fe and Mn, activity is unaffected. Emerson *et al.* conclude that the Fe^{2+} and Mn^{2+} in MndD or HPCD do not need to assume the 3+ oxidation state in the course of catalysis and that they may convey a reducing equivalent between catechol and O_2 without acquiring it. Nonetheless, it is interesting to ponder why the Fe^{3+} state of MndD is much less active than the Fe^{2+} version, if redox activity is not essential, and given that Fe^{3+} has the same d^5 electronic configuration as Mn^{2+}, which does

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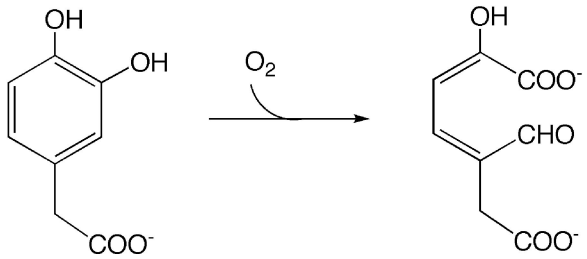

Scheme 1. Overall reaction catalyzed by MndD and Fe-HPCD. An H^+ is also released.

support activity. This beautifully matched pair of enzymes with the same reaction but different native metal ions may have more to teach us still.

Emerson *et al.*'s (3) combination of spectroscopy, enzymology and x-ray crystallography shows that O_2 activation need not depend on the metal ion's redox activity. This flies in the face of the metal-centered universe of chemistry many bioinorganic chemists imagine, but the metal is likely still important for spin reconciliation and transmission of electron density between catecholate and O_2. Binding to the metal ion also stabilizes deprotonated states of the catechol and positions catechol and O_2 adjacent to one another, but Emerson *et al.* present a strong and fascinating argument that a discrete oxidized Fe or Mn intermediate is not needed for reaction. It is the catecholate, not the metal ion, that reduces O_2. The metal ion is not oxidized, but rather reduced, as it were, to a wire.

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