stimuli not at an optimal orientation, the neural response decreased and eventually disappeared completely when the stimulus was in an orientation orthogonal to the preferred orientation. Essentially, Thompson et al. reveal that there are two competing processes—the first is a local decrease in tissue oxygenation due to the increased metabolic demand of the activated neurons; the second is a delayed influx of larger amounts of oxygen into tissue comprising both the activated cortical orientation columns and neighboring regions.

The correspondence between increased neural activity and an “early dip” in tissue oxygenation provides strong evidence that activated neurons do indeed, at least in part, satisfy their increased metabolic demand by increased consumption of oxygen. The delayed increase in tissue oxygenation reflects what has been termed “the overwa-delayed increase in tissue oxygenation resulting from increased consumption of oxygen. The activated neurons do indeed, at least in part, satisfy their increased metabolic demand affected by neural activity and an “early dip” in tissue oxygenation, which results in a “hyperemic overshoot.” In this regard, the Thompson et al. work is a powerful and direct confirmation of the interpretation of previous optical imaging spectroscopy and MRI studies (11).

Perhaps it seems that we are ready to close an important chapter on neuronal metabolism research. The Thompson et al. work, however, reveals that there are still major gaps in our understanding of neuronal metabolism, with important consequences for interpreting BOLD IMRI signals and preventing the reliable comparison of signals between different brain regions. We need to elucidate the relationship between changes in the intensity and duration of stimulation, the resultant changes in neuronal activity, and associated changes in metabolic demand. Next, we need to determine how neural activity and metabolic demand affect the magnitude and timing of the hemodynamic response and of BOLD IMRI measurements.

The Thompson et al. work is an important confirmation of a hypothesis about the metabolic activity of activated neurons. Furthermore, their study helps to elucidate the intrinsic signals underlying BOLD IMRI measurements. However, much work remains to be done before we fully understand the relationship between BOLD measurements and the neural interactions underlying them. As a starting point, we could do with an influx of more research funds!

References

BIOCHEMISTRY

How Iron Activates O₂

Julie A. Kovacs

Several critical processes in biology involve dioxygen (O₂), and most of these processes are promoted by transition-metal ions. Dioxygen serves, for example, as the electron sink that drives the conversion of electrochemical to chemical energy in our bodies. It is also the oxidant and/or oxygen atom source for the biosynthesis of important biomolecules, including DNA, serotonin, fatty acids, and steroids, and for the breakdown of drugs and other foreign substances in our livers. Reports on pages 1039 and 1037 of this issue (1, 2) shed light on some of the intermediates that may be involved in reactions promoted by nonheme iron enzymes.

Dioxygen oxidations are thermodynamically favored (they release energy), but are slow in the absence of a catalyst. Reduced transition-metal ions are ideally suited as catalysts for O₂ oxidation reactions. They are highly reactive with O₂ to afford potent metal-containing oxidants, such as iron-superoxo (Fe(III)-O₂⁻), iron-peroxo (Fe(III)-O₂²⁻), or iron-oxo (Fe(IV)=O and Fe(Ⅴ)=O) species. Until now, there was only indirect evidence for the involvement of any of these intermediate metal-oxygen species in reactions catalyzed by nonheme iron enzymes (3–7). Karlsson et al. (1) and Rohde et al. (2) now reveal the structures of two possible intermediates that may be involved in catalysis by nonheme iron enzymes.

Mechanistic pathways in metalloenzymes (enzymes that rely on transition metals for their catalytic activity) are determined by the electronic and geometric properties of the metal ion and by the surrounding protein environment. The electronic properties of the metal ion are tuned by the coordinated ligands (atoms or molecules bound to the metal) and are subtly altered by hydrogen-bonding interactions with the protein or interactions with nearby metal ions.

For example, replacing the histidine ligand in hemoglobin with a cysteine and placing the iron ion and its coordinated ligands in a slightly less polar protein environment in cytochrome P450 changes the system from a dioxygen carrier to one that activates O₂ and catalyzes the oxidation of unactivated hydrocarbons. If the porphyrin ligand in cytochrome P450 is replaced with four histidines and the metal ion placed closer to the protein surface in superoxido reductase (8, 9), the enzyme no longer activates O₂; rather, it reduces superoxide (O₂⁻, a dangerous cellular toxin) to H₂O₂.

A lot of what we know about the intermediates involved in dioxygen activation comes from studies of heme iron enzymes (those that contain iron in a porphyrin cavity). Examples include horseradish peroxidase and cytochrome P450 (10–12) and their synthetic analogs (13). (Synthetic analogs mimic just the metal ion and its coordination environment, providing greater molecular-level detail because the molecules are smaller.) The conjugated porphyrin ligand governs the pathways by which O₂ is activated. It makes low-spin states—an electronic arrangement in which most or all electrons on the metal ion are paired—accessible and stabilizes highly oxidized iron intermediates.

The ligand environment in mononuclear nonheme iron enzymes, such as isopenicillin N synthase (4) and naphthalene dioxygenase, usually consists of a 2-His-1-carboxylate triad (14). The geometry is more flexible than that of heme iron enzymes, allowing for a more diverse chemistry. Low-spin states are less accessible in nonheme iron enzymes, and the formation of two accessible (labile or vacant) sites adjacent to one another is possible. These structural and electronic differences open up reaction pathways that are unavailable to heme iron (3–7). However, in general, the more reactive an intermediate, the more difficult it is to observe. As a result, very few of the intermediates shown in the fig-
ure have been structurally characterized (10).

The widely accepted mechanism (12) of \( \text{O}_2 \) activation by heme iron enzymes (10–12) involves the initial formation of an Fe\( ^{\text{II}} \)–O\( _2 \) (or Fe\( ^{\text{III}} \)–O\( _2 \)) species (intermediate A), which converts to an end-on Fe\( ^{\text{III}} \)–O\( _2 \)–O species (intermediate B) upon the addition of an electron (step 1 in the figure). Alternatively, a side-on peroxide (intermediate E) may form (step 2). The first observation of a side-on intermediate (A or E) by x-ray crystallography is reported by Karlsson et al. in this issue (1).

A and E are the only intermediates that can activate both oxygen atoms of \( \text{O}_2 \) equally, thus favoring dioxygenase over epoxidase. This mechanism involves the formation of a nonheme dioxygenase enzyme (intermediate C) and a nonheme iron synthetic analog (intermediate D) upon the addition of an electron (step 1 in the figure). Alternatively, a side-on peroxide (intermediate E) may form (step 2). The first observation of a side-on intermediate (A or E) by x-ray crystallography is reported by Karlsson et al. in this issue (1).

As shown by electronic structure calculations (7), a side-on peroxide is made more reactive by adding a proton (step 3). Synthetic analog chemistry shows that protonation will convert a side-on peroxide (intermediate E) to an end-on hydroperoxide (intermediate B) (5). Intermediate B is believed to act as the key catalytic oxidant in some systems (3, 6, 11). Addition of a proton to the distal oxygen of B polarizes the O–O bond, resulting in its heterolytic cleavage (step 4) to afford a high-valent Fe\( ^{\text{IV}} \)–O\( _2 \) species (intermediate C). If the O–O bond is cleaved homolytically (step 5), the slightly less oxidized Fe\( ^{\text{IV}} \)–O (intermediate D) forms. Intermediates C and D are the other key catalytic intermediates implicated in iron-catalyzed oxidation chemistry.

There has been some skepticism as to whether the high-valent iron-oxo species C and D could form without the support of a porphyrin ligand. If one compares the potential energy surface for a heme and a nonheme iron system undergoing step 4 of the scheme, this reaction appears not to be energetically favored for mononuclear nonheme iron systems (6). Borovik and colleagues showed recently that an oxidized Fe\( ^{\text{III}} \)–O can be stabilized in a mononuclear nonheme environment by hydrogen bonds (15). In this issue, Rohde et al. (2) report that Fe\( ^{\text{IV}} \)–O can also form in a nonheme iron synthetic analog. Mechanistic studies imply that an Fe\( ^{\text{IV}} \)–O intermediate serves as the active catalyst in some nonheme iron systems (3). Whether Fe\( ^{\text{IV}} \)–O really forms and can be observed in a nonheme environment remains to be seen.

Although the mechanistic details of biological nonheme iron-promoted dioxygen activation are still being “ironed out,” the observation of highly reactive intermediates reported in this issue (1, 2) provides clues regarding preferred pathways. The two studies show that both protein chemistry and synthetic analog chemistry play vital roles in unraveling the molecular-level details of these critical biological reactions.

References and Notes


Mechanistic pathways available to iron in biology. Reduced iron (Fe\( ^{\text{II}} \)) can react with oxygen to form potent biological oxidants. The molecular details of these reactions are revealed by probing the structure of metalloprotein intermediates or of synthetic analogs. Proposed mechanistic pathways and intermediates are shown, with those highlighted in this issue shaded. L, ligand.

**Imposing Specificity on Kinases**

Mark Ptashne and Alexander Gann

The mitogen-activated protein kinase (MAPK) signaling cascades transmit signals from the cell surface to its interior. There are at least four MAPK cascades in yeast, and in response to a specific extracellular signal (different in each case), one of these cascades “fires.” Each cascade contains three kinases, and sometimes a given kinase (for example, Ste11) is a component of more than one cascade (see the figure). Moreover, despite the fact that each cascade responds to a different signal, several of the cascades are triggered by a common kinase (Ste20). How do different combinations of kinases generate disparate responses? These kinases all have essentially the same active site, which phosphorylates serine or threonine residues in target proteins. How is specificity imposed on these enzymes, that is, how does each kinase choose its correct target protein? How difficult is it to evolve separate pathways using common elements?

On page 1061 of this issue, Park et al. (1) explore these matters by analyzing two MAPK cascades in yeast. One is involved in yeast mating, the other in the response to high salt concentrations in the medium (the osmolarity response). In each case, the three members of the respective cascade are attached to a protein scaffold—Ste5 in the mating cascade, Pbs2 in the osmolarity cascade. Park et al. show that even artificial, and rather loose, tethering of the appropriate kinases to a scaffold suffices for function. They also show that cells bearing a hybrid scaffold (constructed by the authors) elaborate an osmolarity response to a mating signal. These find-