ent them. The underlying idea is simple. Metallic carbon nanotubes can withstand very high current densities (10^9A/cm^2), but at high enough currents the nanotubes will burn up in air. In this way, metallic tubes can be removed selectively from SWNT ropes. Individual nanotube shells can also be removed, one at a time, from multiwalled nanotubes (MWNTs) (*2*). The use of shell-by-shell breakdown of an MWNT to determine the electrical properties of its individual shells is also demonstrated (*2*). By taking advantage of the $1/d_t$ dependence of the band gap of semiconducting shells, FETs with desired band gaps can be produced.

Collins *et al.* (*2*) first use a gate electrode to effectively deplete the electrical carriers (electrons or holes) from the semiconducting tubes within a SWNT rope. The metallic SWNTs within the rope can then be destroyed by current-induced oxidation, leaving the carrier-depleted semiconducting tubes, which carry no current, intact. To fabricate dense arrays of FETs, the SWNT ropes are deposited on an oxidized Si wafer, which also serves as the back gate. An array of source, drain, and side-gate electrodes is then fabricated lithographically on top of the permanently modified SWNT ropes. The concentration of the tubes is preadjusted so that on the average, there is one SWNT rope bridging the source and drain. The back gate (the wafer itself) is used to deplete the semiconducting tubes, followed by the application of a stress voltage to destroy the metallic tubes in the ropes. Dense arrays of nanotube FETs are thus produced in a self-assembly process. The minigaps and pseudogaps observed in STM/STS studies (*1*) do not seem to impede the processing steps of the FETs (*2*).

Both the low-temperature STS/STM studies (*1*) and the selective current-induced removal of nanotube shells (*2*) provide new ways to study the dependence of the electronic DOS on nanotube diameter and chiral angle. Presently, there is no experimental method for synthesizing carbon nanotubes with a specified chiral angle, although progress has been made with achieving some degree of selectivity of the nanotube diameter by controlling growth process parameters, such as growth temperature, carrier gas mixture, and catalyst selection. A recent breakthrough (*13*) showing how to prepare self-assembled bundles of metallic (10,10) armchair nanotubes offers promise that selectivity of nanotube diameter and chiral angle is possible.

Better knowledge of the dependence of physical properties on chiral angle may some day lead to the utilization of some highly sensitive property to selectively control the chiral angle and diameter in the nanotube growth process, so that desired metallic or semiconducting tubes can be grown at will.

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PERSPECTIVES: BIOINORGANIC CHEMISTRY

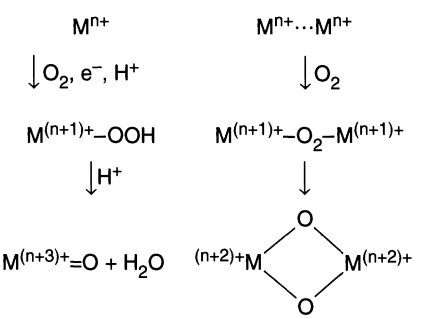
Oxygenase Pathways: Oxo, Peroxo, and Superoxo

Lawrence Que Jr. and Yoshihito Watanabe

Many key metabolic transformations require the controlled oxidation of organic species. These transformations often rely on the activation of molecular oxygen, O_2, by metal ions in the active sites of oxygenase enzymes. At a recent symposium on "Oxygen Activation by Metalloenzymes and Their Models" (*1*), highlighted in this perspective, two themes threaded through a number of the talks: a common oxygen activation scheme and the importance of O_2 in posttranslational modification of proteins.

Oxygen activation can occur at mononuclear heme (porphyrin) sites, nonheme monoiron and diiron sites, mononuclear and dinuclear copper sites, and even at a heterodinuclear heme-copper site. Despite this diversity of active sites, a common mechanistic hypothesis for oxygen activation is emerging. In this unified scheme, oxygen first binds to a reduced metal center; a metal-peroxo intermediate is then formed, followed by O-O bond cleavage to form a high-valent metal-oxo oxidant that carries out substrate oxidation (see the first figure). The extent to which the catalytic cycle of an enzyme (and its corresponding model compounds) follows this mechanism varies from enzyme to enzyme.

The heme enzyme cytochrome P450 is one of the most widely studied systems in bioinorganic chemistry. A high-valent iron-oxo species has generally been thought to be the oxidant responsible for P450-catalyzed oxygenation, but the possibility that an iron-peroxo species may also be involved in some reactions has been raised. For example, Wonwoo Nam (Ehwa Womens University, Seoul, Korea) presented evidence that synthetic iron complexes of highly halogenated, electron-deficient porphyrins catalyze oxygenation reactions through either iron-peroxo or iron-oxo species (*2*). To shed further light on this question, Yoshihito Watanabe (Institute for Molecular Science, Okazaki, Japan) used site-directed mutagenesis to redesign the oxygen carrier myoglobin into a P450-like monooxygenase that can catalyze olefin epoxidation (*3*). Kinetic studies showed that the formation of a high-valent iron-oxo intermediate was the rate-determining step, thus excluding the Fe(III)-OOH (peroxo) intermediate as the oxidant under these conditions. Further evidence comes from studies by Brian Hoffman (Northwestern



Toward a unified mechanism. Scheme for oxygen activation at metalloenzyme active sites.

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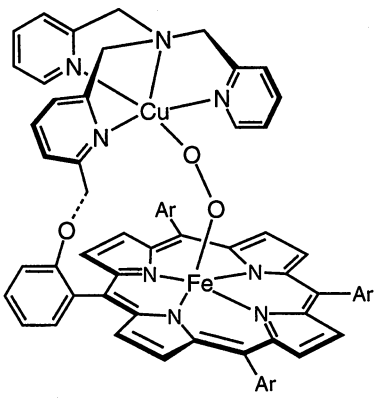
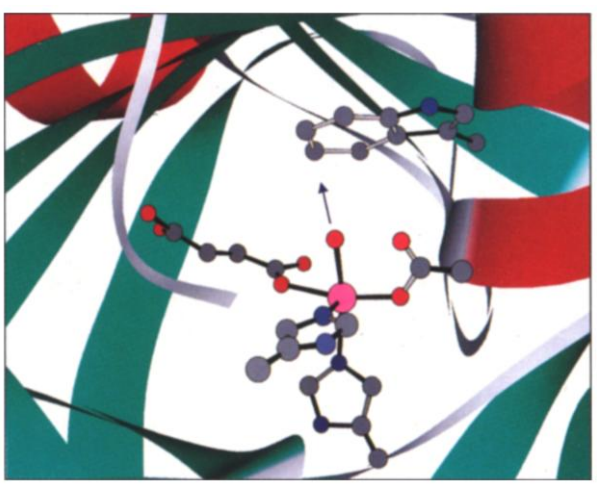
University, Evanston, IL) and co-workers, who have used a novel radiolytic approach to introduce an electron into the reduced P450-dioxygen complex to generate the Fe(III)-OOH (peroxo) intermediate of cytochrome P450 at cryogenic temperatures in the presence of its substrate, *d*-camphor. Brief annealing to around 200 K converted this intermediate to the product complex, showing that the intermediate was catalytically competent despite being generated under these unusual conditions. Hoffman *et al.* monitored the fate of the C-H hydrogen abstracted from the substrate by the oxidant and concluded that the high-valent iron-oxo species must be the active species in this case, although it was not observed directly (*4*). The results are consistent with recent x-ray crystallographic evidence suggesting an iron-oxo species as the cryogenically generated active oxidant of cytochrome P450 (*5*).



Learning from model systems. Proposed structure for Cu-O-O-Fe intermediates of synthetic model systems for the heme/copper dinuclear site of cytochrome oxidase.

Synthetic model systems for the heme/copper dinuclear site of cytochrome oxidase were presented by James Collman (Stanford University, Palo Alto, CA), Kenneth Karlin (Johns Hopkins University, Baltimore, MD), and Yoshinori Naruta (Kyushu University, Fukuoka, Japan). In these models, synthetic porphyrins are tethered to a copper-binding domain. Collman demonstrated clean electrocatalytic reduction of O_2 to water (*6*). Karlin and Naruta presented spectroscopic evidence for the formation of Cu(II)-O-O-Fe(III) intermediates at –78°C (see the second figure) (*7*, *8*). Naruta further showed that the lifetime of the peroxo species could be decreased substantially by introducing an axial nitrogen ligand on the iron porphyrin, which converts the iron center from high spin to low spin. The dependence of the stability of the peroxo species on the spin state may hold the key to efficient oxygen activation (reduction) in this enzyme.

Related metal-peroxo and high-valent metal-oxo species have also been proposed in the oxygen activation mechanisms of metalloenzymes that do not have heme active sites. Our understanding of such nonheme enzyme intermediates has been greatly enhanced by trapping analogous model complexes with ingeniously designed ligands at low temperature. Hideki Masuda (Nagoya Institute of Technology, Nagoya, Japan) and Jean-Jacques Girerd (Université Paris-Sud, Orsay, France) described the properties of metastable mononuclear Fe(III)-OOH complexes of nonporphyrin ligands designed to model putative intermediates in nonheme mono-iron enzymes (*9*). Stephen Lippard [Massachusetts Institute of Technology (MIT), Cambridge, MA] and William Tolman (University of Minnesota, Minneapolis, MN) discussed the use of sterically bulky carboxylate ligands to synthesize carboxylate-bridged di-iron(II) complexes (*10*, *11*). These complexes react with O_2 to form diiron(III)-peroxo or iron(III)-iron(IV) species that may correspond to intermediates in the catalytic cycles of nonheme diiron enzymes such as methane monooxygenase and ribonucleotide reductase (RNR). Shinobu Itoh (Osaka City University, Osaka, Japan) and Masatatsu Suzuki (Kanazawa University, Kanazawa, Japan) found that high-valent bis(μ-oxo)dicopper and dinickel complexes react with aliphatic C-H bonds on the metal ligands (*12*, *13*). Furthermore, the dicopper(III) complexes could oxidize externally added substrates such as cyclohexadiene. Such biomimetic studies shed light on the electronic and reactivity properties of putative enzyme intermediates.



Nonheme iron enzymes. Proposed attack by an iron(IV)-oxo intermediate of a Trp residue in TfdA.

Genomics and proteomics promise to be important tools in biology, but the not infrequent occurrence of posttranslationally modified amino acid residues at metalloenzyme active sites should sound a cautionary note for their use in predicting structures. Prime examples are RNR and galactose oxidase (GO), both of which have catalytically essential tyrosyl radicals. The best studied RNR, that from *Escherichia coli,* generates its tyrosyl radical through the reaction of O_2 with the nonheme diiron centers of the R2 protein (a component of RNR). JoAnne Stubbe (MIT, Cambridge, MA) discussed the complexities of the corresponding yeast enzyme (*14*). Y2 is the yeast protein that corresponds to R2, but tyrosyl radical formation on Y2 requires the addition of a related Y4 protein, perhaps to provide the needed electron for O_2 activation.

GO is a copper enzyme that catalyzes the oxidation of alcohols to aldehydes. The Cu(II) center is coordinated to a Tyr radical that is cross-linked to a Cys residue, and together they act as a two-electron acceptor for the reaction. In studies of the highly purified precursor protein, David Dooley (Montana State University, Bozeman, MT) has established that the assembly of the cross-linked Tyr-Cys unit is a self-processing reaction that requires only protein, Cu(II), and oxygen (*15*). Formation of the copper-tyrosinate complex may thus be sufficient to activate this enzyme for reaction with oxygen. Atomic resolution structures of the native and precursor proteins by Carrie Wilmot, Simon Phillips, and co-workers (University of Leeds, Leeds, UK) show that substantial protein structural changes are required to form the active enzyme. The capability for self-processing by exploiting metal ions and oxygen to produce new cofactors may represent a key step in enzyme evolution.

A new example of post-translational modification was observed for the enzyme TfdA by Lawrence Que (University of Minnesota, Minneapolis, MN) and Robert Hausinger (Michigan State University, East Lansing, MI) (*16*). TfdA is an α-keto acid–dependent nonheme iron enzyme involved in the biodegradation of the herbicide 2,4-D. O_2 can still be activated even in the absence of the substrate 2,4-D in the presence of the α-ketoglutarate cosubstrate, generating an intense blue chromophore. Raman evidence suggests that an active site tryptophan residue is hydroxylated by a proposed high-valent iron-oxo species (see the third figure).

The mechanistic scheme in the first figure thus appears to apply also to this class of monoiron enzymes.

Lastly, Ninian Blackburn (Oregon Graduate Institute of Science and Technology, Beaverton, OR) elaborated on a new mechanism for oxygen activation by the dicopper enzymes peptidylglycine α-amidating monooxygenase (PAM) and dopamine β-monooxygenase (DBM). Crystallography has shown that the two copper centers of PAM are separated by 11 Å in a solvent-accessible cavity (*17*), a gap too large for efficient electron transfer needed to generate a putative Cu(II)-OOH oxidant. The crystallographers suggested that the substrate occupies this cavity and serves as a "wire" between the two copper centers, but Blackburn's spectroscopic studies suggest that the energetic cost of the required reorganization would limit the efficiency of electron transfer. Instead, he has proposed a novel superoxide channeling mechanism (*18*), where O_2 is first reduced to superoxide at one copper and then channels through the solvent-filled cavity where it reacts with the second copper to form the hydroperoxo intermediate. This mechanism, in which superoxide carries both the electron and the coupled proton, represents a new paradigm for monooxygenation reactions at copper centers in proteins.

The symposium stimulated lively discussions among the participants and emphasized the richness of the chemistry associated with oxygen activation at metal centers. A diverse set of techniques has been used to probe the behavior of enzymes and model systems, and much progress has been made, particularly in our understanding of heme enzymes. Future challenges lie in elucidating the corresponding mechanistic details in nonheme enzymes and determing what adaptation Nature has devised to facilitate oxygen activation at such active sites.

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PERSPECTIVES: RNA STRUCTURE

Pulling on Hair(pins)

J. M. Fernandez, S. Chu, A. F. Oberhauser

A broad range of single-molecule techniques now permit direct observation of the activity of single ion channels (*1*), protein and RNA enzymes (*2*, *3*), molecular motors (*4*, *5*), and even larger macromolecular assemblies (*6*). A shared characteristic of all these single-molecule observations is a thermally driven all-or-none discrete transition between conformations. We have come to think that this all-or-none behavior depends on the molecule's having a very large number of interacting atoms, which generate highly cooperative conformational changes. In this issue of *Science* (page 733), Liphardt and colleagues have discovered that short RNA hairpins placed under a stretching force undergo all-or-none discrete transitions in length, which follow a time course strikingly similar to those of transitions observed in ion channels and enzymes (*7*) (see the figure).

RNA is more than a mere messenger: It can fold into three-dimensional structures—ribozymes—that are capable of enzymatic activity (*8*). The self-assembly of RNA enzymes is simpler than that of proteins. For example, in proteins, most secondary structures depend on the global amino acid sequence and are not independently stable, whereas RNAs assemble in a hierarchical manner. Secondary structures such as hairpins, bulges, and three-helix junctions form quickly into stable entities. Once formed, these structures begin a slow dance in search of the final tertiary contacts. These hierarchies make the study of RNA folding a more tractable problem than protein folding. Nevertheless, the folding landscape of RNA enzymes is a complex collection of multiple pathways and transient states that would be difficult to discern with bulk studies. Single-molecule studies, on the other hand, can follow the individual time trajectories of folding and unfolding dynamics.

The work of Liphardt *et al.* elegantly demonstrates these advantages. These investigators used optical tweezers to apply a small force to individual segments of RNA secondary structures suspended between two polystyrene beads. The laser tweezers trap the beads and stretch the RNA structures with a force that can be finely controlled in the piconewton range. Using this approach, they probed the folding of "a simple RNA hairpin, a molecule containing the three-helix junction, and the P5abc domain of the *Tetrahymena* ribozyme" (*7*). The study of the stability of these structures along a well-defined reaction coordinate, the end-to-end distance of the molecules, illuminates how RNA makes possible the formation of structures with proteinlike qualities.

For example, under a stretching force of 14 pN, the RNA hairpin undergoes rapid cycles of all-or-none extension-contraction events of ~18 nm. If the force is increased by only a fraction of a piconewton, the hairpin remains in the extended form, and a small reduction in the force causes the hairpin to remain folded. All these effects can be simply described by assuming that the applied force does mechanical work on the hairpin and changes the height of the activation-energy barrier for the unfolding/folding reaction, as described initially by Bell (*9*). At a critical force, when the unfolding and folding rates are equal (for example, 14 pN), the hairpin is observed to spend equal time in each state. Furthermore, dwell times were found to be exponentially distributed, indicating that the conformational transitions lacked memory (a Markovian process).

The Markovian all-or-none kinetics and the steep force dependence of the dwell times observed for a simple RNA hairpin are hallmarks of ion-channel kinetics. What is going on here? Similarly to the effect of a force, the membrane electric field does work on the ion-channel structure ($W = zeV$), altering the height of the activation-energy barrier and changing the rates exponentially with the applied voltage. At a given membrane potential, the opening and closure rates become equal, and the channel is observed to spend equal times in both states. Hence, the mechanisms generating the kinetics and voltage dependence of an ion channel are very similar to those of a hairpin under a stretching force (see the figure).

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