Although the reader should not be totally convinced that these models are correct, they certainly merit additional testing. Two very recent studies (Luo et al., 2005, Partridge et al., 2005) provide additional mutagenesis evidence that appears to support the model for the resting state, but the first study argues that homomeric clustering is not vital for activation. One daring hypothesis-based test would be to convert the TM region to a water-soluble analog by mutating side chains predicted to be exposed to lipid alkyl chains to hydrophilic residues that occur frequently in coiled-coils. With a bit of luck, these analog structures could then be solved, perhaps with some or all of the soluble domains attached.

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Allosteric Control of O_2 Reactivity in Rieske Oxygenases

Oxygen is Nature's perfect reagent. On one hand, it is potentially a very strong oxidant. On the other hand, this potential is caged because the two highest energy valence electrons of the O_2 molecule are unpaired. As a result, O_2 is relatively unreactive with most other molecules, as almost all of these have paired electrons. Consequently, by modulating the properties of the O_2 valence electrons, Nature can generate a reactive species under controlled conditions, catalyzing difficult reactions while still rigorously enforcing specificity. Special sets of enzymes termed oxygenases and oxidases have evolved to perform this task.

The report by Dobbek and colleagues in this issue of *Structure*, describing the structural basis for the regulation of the O_2 activation in the 2-oxoquinoline 8-monooxygenase oxygenase (OMO) component (Martins et al., 2005), adds a novel strategy to a remarkably varied set of approaches employed by Nature to regulate both when and where O_2 activation occurs. Like so many other aspects of modern biological science, the proposed mechanism has it roots in the mechanism of hemoglobin, ironically a protein specifically designed to bind and transport O_2 without activating it. In this protein paradigm, allosteric interactions transmitted across a subunit boundary generate the cooperative binding of O_2 and allow it to be regulated through binding of proton and organic-phosphate "effectors." In the case of OMO, a subunit boundary is again postulated to transmit allosteric effects. However, the effects originate from a change in the redox state of a Rieske Fe-S cluster and are transmitted across the interface to a mononuclear iron site where O_2 both binds and is activated. Dobbek and colleagues hypothesize that the change in Rieske iron-sulfur cluster redox state induces binding of proton to a histidine ligand and that this, in turn, initiates a cascade of shifts in hydrogen bonding interactions in a network that extends through the interface. Ultimately, these shifts unmask a coordination site on the mononuclear iron, which then binds and activates O_2. In this way, oxygen activation is allosterically controlled, occurring only when the two electrons necessary to satisfy the stoichiometry of the enzyme's monooxygenase reaction [one from the Rieske cluster and the other from the mononuclear Fe(II)] are available. Among its many features, this proposal would account for Nature's choice of a Rieske cluster instead of one of the more common type of FeS clusters for these oxygenases. Only the Rieske cluster has the readily protonated histidine ligands.

The relevance of the Dobbek mechanism to the broad family of Rieske oxygenases is supported by numerous recent studies. The first X-ray crystal structures of a Rieske-type oxygenase completed by Eklund and

colleagues in 1998 were of naphthalene 1,2-dioxygenase (NDO) (Kauppi et al., 1998), an enzyme that inserts both atoms of oxygen from O_2 into the substrate rather than one as for OMO. Nevertheless, there is nearly perfect structural homology in the vicinity of the metal centers and the subunit interface, showing that there is likely to be a common mechanism for regulation of the oxygen activation. The hydrogen bonding network between the metal centers was identified in the original NDO study, but technical difficulties prevented determination of the structure in two redox states, the pivotal advance reported in the current study. The essential nature of the hydrogen bonding network in NDO was established by site-directed mutagenesis in studies published from the Gibson laboratory (Parales et al., 1999). Finally, redox-related structural changes in the vicinity of the Rieske center (Cosper et al., 2002) and coordination number flexibility of the mononuclear site (Gassner et al., 1993) have emerged from numerous spectroscopic studies.

Recently, we have used single turnover reaction kinetics of reduced NDO and benzoate 1,2-dioxygenase (BZDO) to show that the two electrons present in the metal clusters are both utilized during product formation, implying that the electron transfer event between the centers is critical for catalysis (Wolfe et al., 2001, 2002). Moreover, the use of NO as an O_2 surrogate allowed us to demonstrate that small-molecule access to the mononuclear iron site is regulated by both substrate binding and the redox state of the Rieske cluster. Indeed the presence of allosteric coupling between the Rieske and mononuclear-iron sites was first inferred from an ENDOR spectroscopic study of the NDO-NO complex with specifically deuterated substrates bound in the active site; it showed an 0.5 Å shift in the position of the substrate upon reduction of the 13 Å distant Rieske cluster (Yang et al., 2003a, 2003b). Thus, studies of the enzyme in solution are in perfect agreement with the structural insights that have now emerged from the X-ray crystal structure of OMO.

Oxygenases and oxidases use multiple strategies to regulate O_2 activation, and the current study shows that elements of known strategies are recombined in a novel form in OMO. It is likely that additional elements of regulation and mechanistic features of Rieske oxygenases will emerge from future X-ray and ENDOR studies. For example, it is currently unknown how the mononuclear iron site of Rieske dioxygenases can promote both monooxygenase and dioxygenase chemistry, and the current availability of structures for both types of Rieske oxygenases will permit detailed comparisons. Also, while current information shows how OMO can link a redox reaction to O_2 activation, it does not reveal how the activation process is coupled to substrate binding. As alluded to above, spectroscopic studies clearly show that the probe molecule NO does not bind to the iron in fully reduced NDO prior to substrate addition (Wolfe et al., 2001). If this is also true of O_2, then Nature has devised a mechanism to insure that two electrons *and* substrate are present before O_2 activation. In the case of NDO, the crystal structures of both substrate-free and bound oxygen adducts have been reported (Karlsson et al., 2003). While this may mean that the enzyme binds O_2 differently than NO, this seems unlikely because activation of O_2 without substrate would result in the uncontrolled release of activated oxygen species, which is neither observed in solution nor tolerated in biology.

Reactive oxygen species can be toxic in humans, and oxygen under high pressure is a poison, yet humans and all other aerobic organisms on earth have adapted to, and indeed require, abundant oxygen. Thus, adaptive pressure to regulate oxygen reactivity is relentless. OMO provides a fresh example of the diversity with which Nature has responded to this challenge.

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