

RESEARCH ACTIVITIES IX

Center for Integrative Bioscience

IX-A Molecular Mechanisms of Oxygen Activation by Heme Enzymes

By sharing a common prosthetic group, the heme enzymes such as cytochrome P450s, peroxidases, and catalases catalyze their own unique biological functions; monooxygenation, hydrogen peroxide dependent oxidation, and dismutation of hydrogen peroxide, respectively. Our efforts have been focused on the elucidation of the structure-biological function relationship of these heme enzymes by employing both enzymatic systems including mutants and their model systems.

IX-A-1 Molecular Engineering of Myoglobin: The Improvement of Oxidation Activity by Replacing Phe-43 with Tryptophan

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The replacement of Phe-43 in sperm whale myoglobin (Mb) by a tryptophan residue has been investigated to examine if an electron rich oxidizable amino acid residue in the heme vicinity increases oxidation activities of Mb. F43W Mb exhibits approximately 6- and 8-fold higher V_{\max} values than the wild type in guaiacol and ABTS oxidations, respectively. However, the one-electron oxidation activity for F43W/H64L Mb is less than that of the F43W single mutant because the absence of histidine in the distal heme pocket suppresses the compound I formation. More than 15-fold improvement versus wild type Mb in the rates of two-electron oxidation of thioanisole and styrene are observed by the Phe-43 → Trp mutation. Our results indicate that Trp-43 in the mutants enhances both one- and two-electron oxidation activities (*i.e.* F43W Mb > Wild type Mb and F43W/H64L Mb > H64L Mb). The value of ¹⁸O incorporation from H₂¹⁸O₂ into the epoxide product for the wild type is 31%; however, the values for F43W and F43W/H64L Mb are 75 and 73%, respectively. Thus, Trp-43 in the mutants does not appear to be utilized as protein radical site in the oxidation. Furthermore, compound I of F43W/H64L Mb exhibits an absorption spectrum typical for a ferryl porphyrin radical cation, which is reduced back to the ferric state at the rate of 360 s⁻¹ in the presence of thioanisole. The rate is approximately 10-fold greater than the value for H64L Mb. Our results suggest that a tryptophan in the active site of Mb mutants increases the reactivity of compound I, but the enhanced reactivity is not associated with a stable protein radical formation in the heme pocket. The oxidative protein modification of F43W/H64L Mb observed during the reaction with *m*-chlorobenzoic acid (*m*CPBA) has also been reported.

IX-A-2 Oxidative Modification of Tryptophan-43 in the Heme Vicinity of the F43W/H64L Myoglobin Mutant

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The F43W/H64L Mb mutant was previously constructed to investigate the effects of electron-rich tryptophan residue in the heme vicinity on the catalysis, (Figure 1) and we found that Trp-43 in the mutant was oxidatively modified in the reaction with *m*-chloroperbenzoic acid (*m*CPBA). To identify the exact structure of the modified tryptophan in this study, the *m*CPBA-treated F43W/H64L mutant has been digested stepwise with Lys-C achromobacter and trypsin, and two oxidation products are isolated by preparative FPLC. The close examinations of the ¹H NMR spectra of peptide fragments reveal that two forms of the modified tryptophan must have 2,6-disubstituted indole substructures. The ¹³C NMR analysis suggests that one of the modified tryptophan bears a unique hydroxyl group at the amino-terminal. The results together with Ms/Ms analysis (30 Da increase in mass of Trp-43) indicate that oxidation products of Trp-43 are 2,6-dihydro-2,6-dioxindole and 2,6-dihydro-2-imino-6-oxindole derivatives. (Figure 2) Our finding is the first example of the oxidation of aromatic carbons by the myoglobin mutant system.

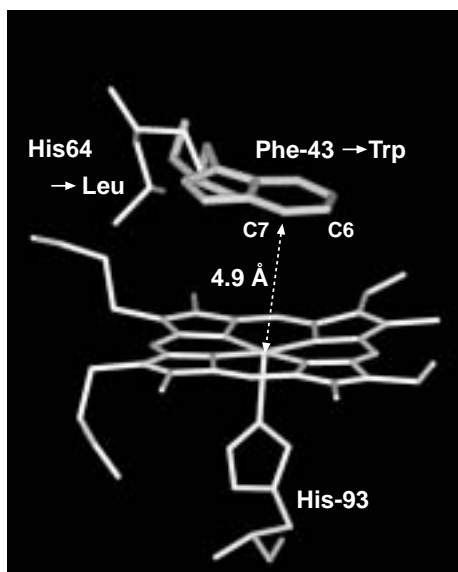


Figure 1. Calculated structure of F43W/H64L Mb using the Insight II molecular modeling program (Biosym MSI, San Diego, CA).

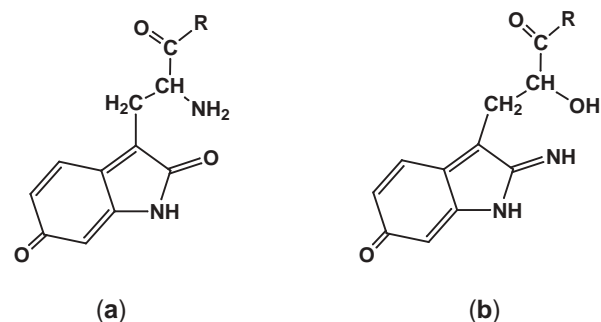


Figure 2. The oxidation products of Trp-43; (a) 2,6-dihydro-2,6-dioxindole and (b) 2,6-dihydro-2-imino-6-oxindole derivatives.

IX-B Model Studies of Non-Heme Proteins

Non-heme proteins play important roles in biological redox processes. Many reactions catalyzed by the non-heme enzymes are quite similar to those by hemoproteins. We are interested in the active intermediates responsible for oxidation and oxygenation by non-heme enzyme, especially the similarity and differences.

IX-B-1 (Catecholato)iron(III) Complexes: Structural and Functional Models for the Catechol-bound Iron(III) Form of Catechol Dioxygenases

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[*J. Inorg. Biochem.* in press]

The metabolic conversion of aromatic compounds to aliphatic compounds is of fundamental importance in biology. Catechol dioxygenases are mononuclear non-heme iron enzymes that catalyze the oxygenation of catechols to aliphatic acids via the cleavage of aromatic rings. These enzymes can be divided into two types: intradiol-cleaving enzymes which break the catechol C1–C2 bond, and extradiol-cleaving enzymes which break the C2–C3 or C1–C6 bond. In the last 20 years, a number of (catecholato)iron(III) complexes have been synthesized and characterized as structural and functional models for the catechol-bound iron(III) form of catechol dioxygenases. This review collects the structural and spectroscopic characteristics and oxygenation activity of (catecholato)iron(III) complexes as structural and functional models for the catechol-bound iron(III) form of catechol dioxygenases.

IX-B-2 Biomimetic Intradiol-Cleavage of Catechols with Incorporation of Both Atoms of O₂: The Role of the Vacant Coordination Site on the Iron Center

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[*Chem. Lett.* in press]

Since Hayaishi *et al.* have revealed that an intradiol-cleaving catechol dioxygenase, pyrocatechase, catalyzes the oxygenation of catechol to muconic acid with incorporation of two oxygen atoms of O₂ (but not of H₂O), the oxygenation mechanisms of catechol dioxygenases have been studied through investigations of model systems as well as the enzymes themselves. However, details of the O₂ insertion and aromatic ring-cleavage reactions are not yet understood. Herein, we report the first example of model system to display intradiol-cleavage of catechols with incorporation of two oxygen atoms of O₂ promoted by iron complexes (Figure 1): [Fe^{III}(³L)(DBC)(DMF)] (**1**, ³L = *N*-(2-hydroxyphenyl)-*N*-(2-pyridylmethyl)benzylamine, DBC = 3,5-di-*tert*-butylcatecholato, DMF = *N,N*-dimethylformamide) and [Fe^{III}(³L)(DBC)Cl](PPh₄) (**2**).

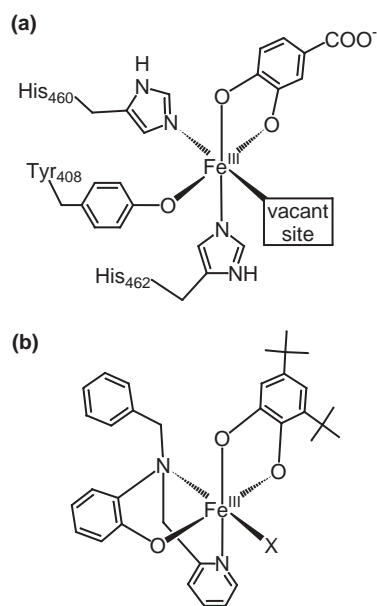


Figure 1. (a) Active site structure of a protocathechuic acid-bound form of protocathechuate 3,4-dioxygenase and (b) complexes **1** (X = Cl) and **2** (X = DMF).

IX-C Aqueous Organometallic Chemistry

Development of water-soluble organometallic catalysts is a worthy endeavor because of potential advantages such as reaction-specific pH selectivity, introduction of new biphasic processes, and alleviation of environmental problems associated with the use of organic solvents. Although the majority of these studies have been carried out with water-soluble organometallic complexes containing water-soluble phosphine ligands, few have utilized organometallic complexes containing water molecules as ligands (*i.e.* organometallic aqua complexes). These organometallic aqua complexes are promising new pH-selective catalysts since their structures drastically change as a function of pH due to deprotonation of the H₂O ligands.

IX-C-1 pH-Dependent Transfer Hydrogenation, Reductive Amination, and Dehalogenation of Water-Soluble Carbonyl Compounds and Alkyl Halides Promoted by Cp*Ir Complexes

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[Organometallics in press]

Recently, we reported a pH-dependent transfer hydrogenation of water-soluble carbonyl compounds with an aqua complex [Cp*Ir^{III}(H₂O)₃]²⁺ (**1**, Cp* = η⁵-pentamethylcyclopentadienyl) as a catalyst precursor and HCOONa as a hydrogen donor. We have extended our study with **1** to that with [(Cp[^]py)Ir^{III}(H₂O)₂]²⁺ (**2**, Cp[^]py = η⁵-(tetramethylcyclopentadienyl)-methylpyridine) and [Cp*Ir^{III}(bpy)(H₂O)]²⁺ (**3**, bpy = 2,2'-bipyridine), since we expect that the ligation of pyridine and bipyridine in **1** could change its catalytic activity due to the change of the Lewis acidity of the iridium ion. Herein, we report preliminary findings of pH-dependent transfer hydrogenation, reductive amination, and dehalogenation of water-soluble carbonyl compounds and alkyl halides with **1**, **2**, and **3** as catalyst precursors and HCOONa and HCOONH₄ as hydrogen

donors. The pH-dependence in these reactions is discussed on the basis of (i) deprotonation processes of the catalyst precursors, (ii) pH-dependent behavior of the hydrogen donors, and (iii) pH-dependent formation of the active catalysts.

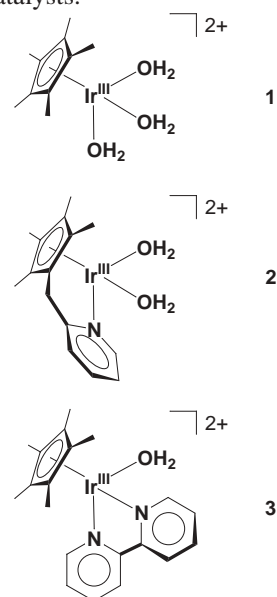


Figure 1. Complexes **1**, **2**, and **3**.