IX-E Electronic Structure and Reactivity of Active Sites of Metalloproteins

Metalloproteins are a class of biologically important macromolecules that have various functions such as oxygen transport, electron transfer, oxidation, and oxygenation. These diverse functions of metalloproteins have been thought to depend on the ligands from amino acid, coordination structures, and protein structures in immediate vicinity of metal ions. In this project, we are studying the relationship between the structures of the metal active sites and functions of metalloproteins.

IX-E-1 Structural Model of Active Site of Protocatechuate 3,4-Dioxygenase: Trigonal Bipyramidal Ferric Aqua Complex with Sterically Hindered New Salen Ligand

FUJII, Hiroshi

Protocatechurate 3,4-dioxygenase (3,4-PCD) has been found in soil bacteria and known to serve as a part of degrading aromatic molecules in nature. The enzyme is classified into an intradiol dioxygenase and contains a ferric iron as a catalytic reaction site. The enzyme cleaves catechol analogies bound to ferric iron site into aliphatic products with incorporating both atoms of molecular. It has been proposed that the enzyme activates an iron bound catecholate to react with an oxygen molecule, but not an iron bound oxygen molecule as most non-heme iron enzymes. Thus, the structure of the ferric iron site has been thought essential to understand the unique reaction of 3,4-PCD. Previous crystal structure of 3,4-PCD from *Pseudomonas putida* reveals a unique trigonal bipyramidal ferric iron site with four endogenous protein ligands (Tyr408, Tyr447, His460, and His462) and a solvent-derived water molecule. To understand the structure-function relationship of 3,4-PCD, there have been attempted over several decades to prepare inorganic model complexes that mimic the ferric iron site of 3,4-PCD, however, no ferric iron complex with the same coordination structure that in the enzyme has been characterized definitively. In this paper, we report the successful attainment to the ferric iron active site of 3,4-PCD by using sterically hindered salen ligand (Figure 1). Characterization of the present model complex reveals the roles of the iron bound water ligand in the enzyme on the unique trigonal bipyramidal structure and the catechol degradation reaction.

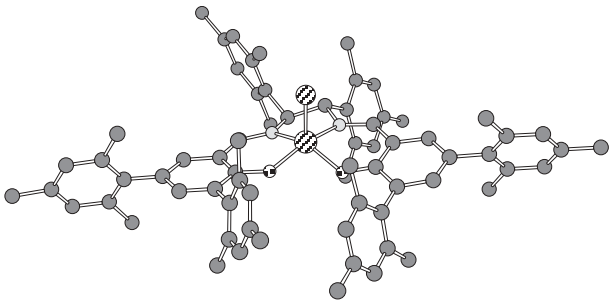

Figure 1. Structure of sterically hindered iron(III) salen complex prepared in this project.

IX-E-2 Synthesis and Characterization of High Valent Iron Porphyrin Complexes as Models for Reaction Intermediates of Cytochrome *c* Oxidase

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Cytochrome c oxidase (CcO) is the terminal oxidase that reduces molecular oxygen to water, coupling with proton pumping across the mitochondrial inner membrane. Since discovery of this enzyme, many structural and functional studies have been done to understand its reaction mechanism. Recent X-ray analyses reveal that this enzyme contains a binuclear center, heme-a_3-Cu_B site, as a reaction site. The binuclear center of the resting enzyme is ferric/cupric form. The binuclear active site is reduced to a ferrous/cuprous form by two electrons from cytochrome c through the Cu_A and heme a site. The ferrous/cuprous form of active site reacts with O_2 to yield an internal dioxygen adduct, intermediate A state, which is further converted to intermediate P and F by the aid of the electrons and protons. Although the intermediates P and F have been studied by resonance Raman and flash-flow absorption spectroscopies, the electronic states of these intermediates are not still clear. To reveal the electronic states of these intermediates and to understand the reaction mechanism of CcO, we have synthesized model complexes of the heme-a_3 site of cytochrome c oxidase. The model complex contains a formyl group at pyrrole-β position to mimic the heme a_3 and mesityl groups to stabilize high valent oxo iron species (see Figure 1). We have succeeded in the preparation of a high valent oxo iron porphyrin complex as a model for the intermediate P by the oxidation of the ferric model complex with mCPBA or ozone.

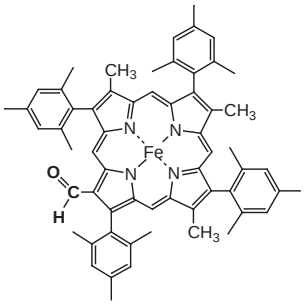

Figure 1. Structure of model complex prepared in this project as models for heme-a and heme-a_3 site of cytochrome c oxidase.

IX-F Molecular Mechanism of Heme Degradation and Oxygen Activation by Heme Oxygenase

Heme oxygenase (HO), an amphipathic microsomal proteins, catalyzes the regiospecific oxidative degradation of iron protoporphyrinIX (heme) to biliverdinIXα, carbon monoxide, and iron in the presence of NADPH-cytochrome P-450 reductase, which functions as an electron donor. Heme oxygenase reaction is the biosynthesis processes of bile pigments and CO, which is a possible physiological messenger. Recent development in the bacterial expression of a soluble form of heme oxygenase has made it possible to prepare in the large quantities for structural studies. In this project, we are studying the molecular mechanism of heme degradation and the oxygen activation by heme oxygenase using various spectroscopic methods.

IX-F-1 A Role for Highly Conserved Carboxylate, Aspartate-140, in Oxygen Activation and Heme Degradation by Heme Oxygenase-1

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Heme oxygenase (HO) catalyzes the oxygen-dependent degradation of heme to biliverdinIXα, CO, and free iron ion via three sequential monooxygenase reactions. Although the distinct active site structure of HO from cytochrome P450 families suggests unique distal protein machinery to activate molecular oxygen, the mechanism and the key amino acid for the oxygen activation have not been clear. To investigate the functionality of highly conserved polar amino acids in the distal helix of HO-1, we have prepared alanine mutants: T135A, R136A, D140A, and S142A, and found drastic changes in the heme degradation reactions of D140A. In this paper, we report the first evidence that D140 is involved in the oxygen activation mechanism in HO-1. The heme complexes of HO mutants examined in this study fold and bind heme normally. The pKa values of the iron bound water and autooxidation rates of the oxy-form are increased with R136A, D140A, and S142A mutations, but are not changed with T135A mutation. As the wild type, T135A, R136A, and S142A degrade heme to verdohemeIXα with H_2O_2 and to biliverdinIXα with the NADPH reductase system. On the other hand, D140A heme complex forms compound II with H_2O_2 and no heme degradation occurs. For the NADPH reductase system, the oxy form of D140A heme complex is accumulated in the reaction and only 50% of heme is degraded. The stopped flow experiments suggest that D140A can not activate iron bound dioxygen and hydroperoxide properly. To investigate the carboxylate functionality of D140, we further replaced D140 with glutamic acid (D140E), phenylalanine (D140F), and asparagine (D140N). D140E degrades heme normally but D140N shows reactivity similar to D140A. D140F loses heme degradation activity completely. All of these results indicate that the carboxylate at position 140 is essential to activate the iron bound dioxygen and hydroperoxide. On the basis of the present findings, we propose an oxygen activation mechanism involving the hydrogen-bonding network through the bridging water and D140 side chain.

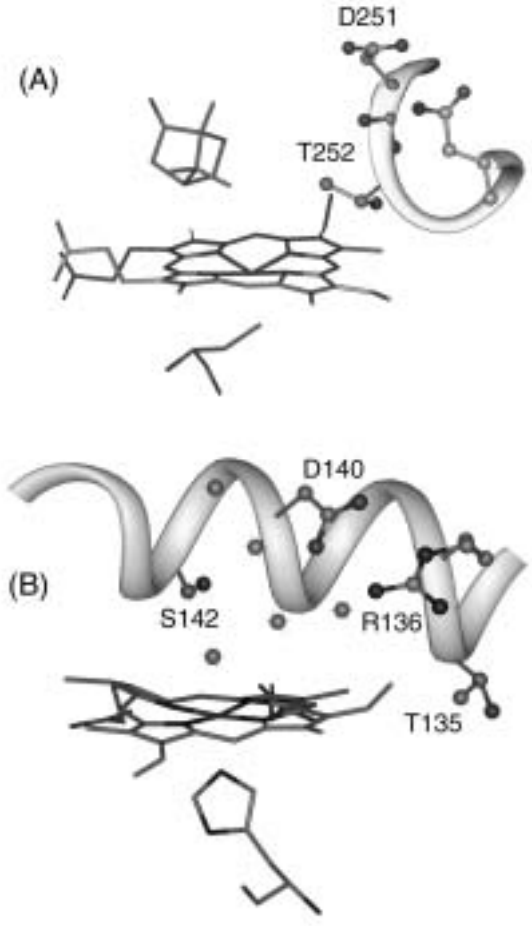

Figure 1. Active site structures of cytochrome P450 (A) and heme oxygenase (B).

IX-F-2 Catalytic Mechanism of Heme Oxygenase Through EPR and ENDOR of Cryoreduced Oxy-Heme Oxygenase and Asp 140 Mutants

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Heme oxygenase (HO) catalyzes the O_2- and NADPH-cytochrome P450 reductase-dependent conversion of heme to biliverdin and CO through a process in which the heme participates both as prosthetic group and substrate. It was proposed that the first mono-oxygenation step of HO catalysis is the conversion of the heme to α-meso-hydroxyheme, through a process in which an electron provided by NADPH-cytochrome P450 reductase reduces the first heme to the ferrous state and molecular of dioxygen binds to form a metastable O_2-bound complex, which then is reduced by a second electron to generate hydroperoxy ferric-HO. It was further thought that the hydroperoxy-ferric HO is the reactive hydroxylating species, rather than a high-valent ferryl active intermediate as in the case of cytochrome P450cam. However, neither the putative hydroperoxo-ferric-HO intermediate nor the a-meso-hydroxyheme product had been detected during physiological HO catalysis until our recent EPR and ENDOR study of oxy-ferrous HO cryoreduced at 77 K. In the present study we have generated a detailed reaction cycles for the first mono-oxygenation step of HO catalysis. We employed EPR and ^{1}H, ^{14}N ENDOR spectroscopies to characterize the intermediates generated by 77 K radiolytic cryoreduction and subsequent annealing of wild-type oxy-HO and D140A, F mutants.

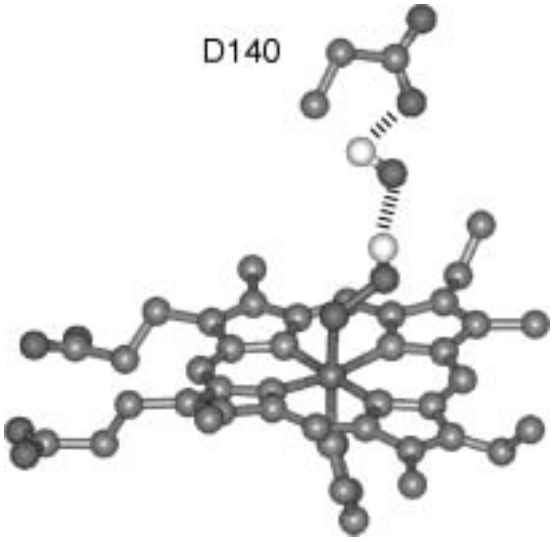

Figure 1. Structure of ferric hydroperoxide intermediate in HO.