IX-F 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 acids, 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-F-1 Trigonal Bipyramidal Ferric Aqua Complex with Sterically Hindered Salen Ligand as a Model for Active Site of Protocatechuate 3,4-Dioxygenase

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Protocatechurate 3,4-dioxygenase (3,4-PCD) has been found in soil bacteria and is known to play a role in degrading aromatic molecules in nature. The enzyme is classified as an intradiol dioxygenase and cleaves catechol analogues bound to the iron(III) site into aliphatic products with incorporation of both atoms of molecular oxygen. It has been proposed that the enzyme does not activate an iron-bound oxygen molecule, but rather induces an iron-bound catecholate to react with O_2. Therefore, knowledge of the structure and electronic state of the iron site is essential to understanding the unique reaction of 3,4-PCD. A previous crystalstructure analysis of 3,4-PCD from Pseudomonas putida revealed a distorted trigonal-bipyramidal ferric iron center with four endogenous protein ligands (Tyr 408, Tyr 447, His 460, and His 462) and a solvent-derived water molecule (see Figure 2). To understand the structure-function relationship of 3,4-PCD, attempts have been made over several decades to prepare inorganic model complexes of 3,4-PCD. However, no iron(III) complex that reproduces the active site of 3,4-PCD has been characterized. We report here the first example of a distorted trigonal-bipyramidal ferric aqua complex with a sterically hindered salen ligand that not only duplicates the active site but also mimics the spectral characteristics of 3,4-PCD.

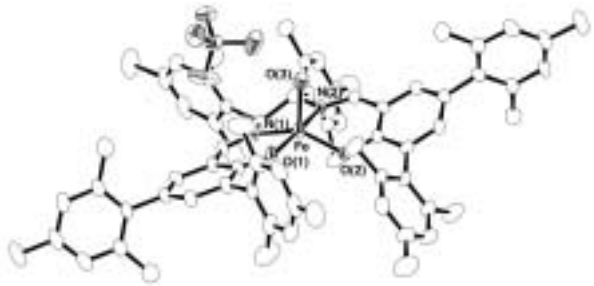

Figure 1. Structure of 3,4-PCD active site model complex prepared in this project.

IX-F-2 ^{13}C-NMR Signal Detection of Iron Bound Cyanide Ions in Ferric Cyanide Complexes of Heme Proteins

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Small molecule axial ligands potentially can serve as useful NMR probes for characterization of environment and electronic structure of prosthetic group in heme protein. In this regard, the diamagnetic ferrous states have been examined thoroughly because of easy signal detection from iron bound small molecule. The ^{13}C-NMR signal of ^{13}CO form of heme protein proves to be sensitive to the nature of the trans amino acid ligand. For the paramagnetic ferric state, cyanide ion would appear to have the greatest potential because of its extremely high affinity to ferric heme iron center. ^{15}N-NMR signals of the iron bound C^{15}N have been detected in a far-downfield region for both iron(III) porphyrin model complexes and heme proteins. However, the ^{15}N-NMR spectroscopy remains ambiguity as a NMR probe since the ^{15}N-NMR shift reflects the nature of both hydrogen bond in the distal side and amino acid ligand in the proximal side. On the other hand, ^{13}C-NMR spectroscopy of the iron bound ^{13}CN has been investigated in less detail. Although ^{13}C-NMR signals of the iron bound ^{13}CN are detectable in a far-upfield region (~ –2500 ppm from TMS) for bis-cyanide iron(III) porphyrin model complexes, extreme line-broadening of the signal seemed to preclude the signal detection in heme proteins and a resonance of the iron bound ^{13}CN for ferric heme protein has not yet been located. During a more extensive ^{13}C-NMR study, we found the ^{13}C-NMR signals of the iron bound ^{13}CN of ferric cyanide complexes of heme proteins and its model complexes at an unexpectedly large upfield region (~ –4000 ppm from TMS). Here, we report the first detection of ^{13}C-NMR signal of the iron bound ^{13}CN in heme proteins such as sperum whale myoglobin(Mb), human hemoglobin(Hb), horse heart cytochrome *c*(Cyt-*c*), and horseradish peroxidase(HRP). This study shows that the ^{13}C-NMR spectroscopy of the iron bound ^{13}CN provides a probe for studying nature of the proximal ligand in ferric heme protein.

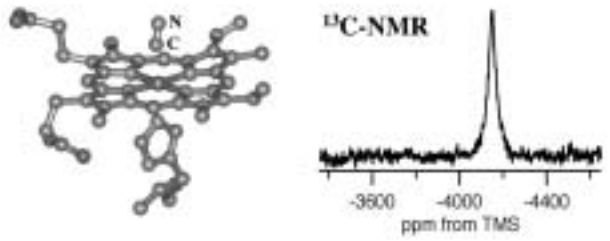

Figure 1. Active site structure of cyanide form of ferric heme protein and its ^{13}C-NMR spcrtrum.

IX-G 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-G-1 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 a-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 meta-stable O_2-bound complex, which then is reduced by a second electron to generate hydroperoxy ferric-HO. It was further thourght 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 charcterize the intermediates generated by 77 K radiolytic cryoreduction and subsequent annealing of wild-type oxy-HO and D140A, F mutants.