VIII-D Elecrtonic Structures and Rectivities of Active Sites of Metalloproteins

Metalloproteins are a class of biologically important macromolecules which 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.

VIII-D-1 Resonance Raman Spectra of Legitimate Models for the Ubiquitous Compound I Intermediates of Oxidative heme Enzymes

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Resonance Raman (RR) spectra are reported for two models of the compound I intermediates of oxidative heme proteins; namely, the imidazole (Im) and 2-methyl-imidazole (2-MeIm) complexes of the ferryl π-cation radical derivative of iron-(5,10,15,20-tetra-mesitylporphyrin), $[O=Fe(TMP^{+\bullet})(Im)]^+$ and $[O=Fe(TMP^{+\bullet})]^+$, which are stablized in dichloromethane solution at –80 °C. The present study yields high quality RR spectra of these complexes and provides the forst opportunity to compare the ν(Fe=O) stretching modes and the structure-sensitive core maker modes for a ferrylporphyrin π-cation radical with the corresponding modes of the neutral parent bearing the same trans-axial ligand. While the observed shifts in the frequencies of the core modes are in agreement with those expected upon formation of the π-cation radical, the results suggest that the isolated effect of macrocycle oxidation on the Fe=O stretching frequency is rather small; the observed shift being only about 4 cm^{-1} to lower frequency.

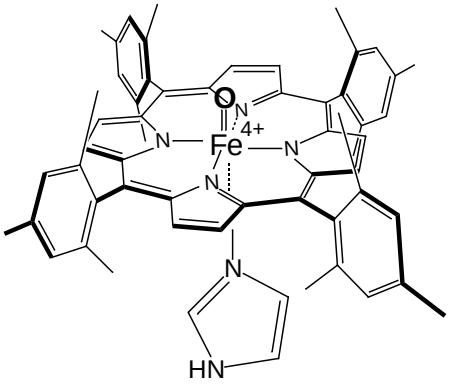

Figure 1. Structure of model complexes of the compounds I of oxidative heme proteins.

VIII-D-2 Spin Distribution in Low-Spin (meso-Tetraalkylporphyrinato)iron(III) Complexes with $(dxz,dyz)^4$ $(dxy)^1$ Configuration. Studies by ^{1}H-NMR, ^{13}C-NMR, and EPR Spectroscopies

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^{1}H-NMR, ^{13}C-NMR, and EPR studies of a series of low-spin (meso-tetraalkylporphyrinato)iron(III) complexes, $[Fe(TRP)(L)_2]X$ where R = n-Pr, c-Pr, and i-Pr and L represents axial ligands such as imidazoles, pyridines, and cyanide, have revealed that the ground-state electron configuration of $[Fe(TnPrP)(L)_2]X$ and $[Fe(TcPrP)(L)_2]X$ is presented either as the common $(dxy)^2$ $(dxz,dyz)^3$ or as the less common $(dxz,dyz)^4$ $(dxy)^1$ depending on the axial ligands. The ground-state electron configuration of the isopropyl complexes $[Fe(Ti\text{-}PrP)(L)_2]X$ is, however, presented as $(dxz,dyz)^4$ $(dxy)^1$ regardless of the kind of axial ligands. In every case, the contribution of the $(dxz,dyz)^4$ $(dxy)^1$ state to the electronic ground state increases in the following order: HIm < 4-Me_2NPy < 2-MeIm < CN^- < 3-MePy < Py < 4-CNPy. Combined analysis of the ^{13}C and ^{1}H NMR isotropic shifts together with the EPR *g* values have yielded the spin densities at the porphyrin carbon and nitrogen atoms. Estimated spin densities in $[Fe(TiPrP)(4\text{-}CNPy)_2]^+$, which has the purest $(dxz,dyz)^4$ $(dxy)^1$ ground state among the complexes examined in this study, are as follows: meso-carbon, +0.045; β-pyrrole carbon, +0.0088; α-pyrrole carbon, –0.00026; and pyrrole nitrogen, +0.057. Thus, the relatively large spin densities are on the pyrrole nitrogen and meso-carbon atoms. The result is in sharp contrast to the spin distribution in the $(dxy)^2$ $(dxz,dyz)^3$ type complexes; the largest spin density is at the α-pyrrole carbon atoms in bis(1-methylimidazole)(meso-tetraphenylporphyrinato)-iron(III), $[Fe(TPP)(1\text{-}MeIm)_2]^+$, as determined by Goff. The large downfield shift of the meso-carbon signal, ä +917.5 ppm at –50 °C in $[Fe(TiPrP)(4\text{-}CNPy)_2]^+$, is ascribed to the large spin densities at these carbon atoms. In contrast, the large upfield shift of the β-pyrrole carbon signal, –293.5 ppm at the same temperature, is caused by the spin polarization from the adjacent meso-carbon and pyrrole nitrogen atoms.

VIII-D-3 Post-Assembly Insertion of Metal Ions into Thiol-Derivatized Porphyrin Monolayers on gold

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The insertion of metal ions into thiol-derivatized free base porphyrin monolayers pre-assembled on gold has been conducted by refluxing the metal ion solution in which the monolayer-coated gold electrode was immersed. The extend of the metal insertion was estimated from the decrease in the N1s peaks in X-ray photoelectron spectra (XP spectra) assigned to the pyrrole nitrogen which binds a hydrogen atoms. The insertion of Co(II) was completed by refluxing for 3 hr. Although the extent of the metal insertion for the same reflux time depends on the metal ion used, the insertion of several ions including Mn(II), Fe(II), Ni(II), Cu(II) and Zn(II) was possible. Besides XP spectra, the metal insertion was confirmed by the electrocatalytic activity of the monolayers for the reduction of molecular oxygen. The structural characterization has proved that the monolayer is stable during the reflux: neither desorption nor change in the orientation of the porphyrin molecules took place. Compared to the commonly used self-assembly of the pre-metalated porphyrin, this post-assembly metal insertion method has an advantage because neither intra nor intermolecular coordinations of the thiol functionality to the central metal ion take place, thus avoiding the unexpected disorder in the monolayer such as the formation of a multilayer, the blocking of the electrocatalytically active metal ion and loss of the anchoring functionality or thiol.

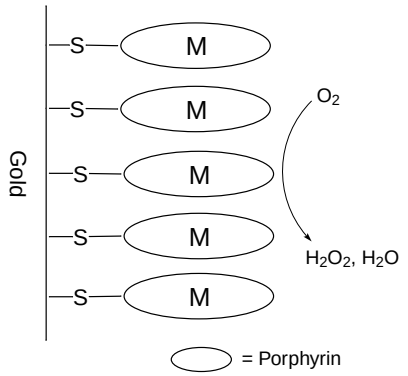

Figure 1. Porphyrin monolayer formed by the self-assemble on gold surface.

VIII-D-4 Electron Spin-Echo Envelope Modulation Spectral properties of Amidate Nitrogen Coordinated to Oxovanadium(IV) Ion

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[*Chem. Lett.* 198 (2000)]

Increse evidence shows that vanadium palys a variety of roles in biological systems. For instance, a class of haloperoxidase requires vanadium for their enzymatic activities. Vanadium is also know to have beneficial insulin-mimetic activities, and some vanadium complexes are studied as a candidate for an orally-active anti-diabetic agent. These findings have stimulated interests in the interactions of vanadium with biological substances such as an amnio acids, peptides, and proteins. In the stduies on this subject, interests are often focused on carboxylate, imidazole and amino groups for vanadium-coordinating groups. However, recent studies have shown that amido group can undergo reprotonation/coordination reaction even at physiological conditions when an anchoring group is present. Therefore, it is possible that vanadium-amidate bonding actually ocuurs and plays some roles in biological systems. For characterization of vanadium(IV) cooridnation environments, electron spin-echo envelope modulation (ESEEM) spectroscopy is suited. It has been demonstrated that ESEEM results not only reveal the presence or absence of nitrogen nuclei coordinated to VO^{2+} ion (and possibly the number of the coordinating nitrogen atoms), but allow identification of equatorial nitrogens based on the emprirical correlation between the type of the nitrogen and the ^{14}N hyperfine coupling (HFC) parameter. However, neither the HFC parameters nor the nuclear quadropole coupling (NQC) pararmeters are known for vanadium-coordinated amidataed nitrogens. Here we report the first ESEEM results for a structurally-characterized VO^{2+}-amidate complex.

VIII-D-5 Newly Designed Iron-Schiff Base Complexes as Models of Mononuclear Non-Heme Iron Active Sites

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High valent iron-oxo species have been suggested as the active intermediates for catalytic oxygenation reactions by iron-containing oxygenases. In the reaction mechanisms of heme and binuclear non-heme iron enzymes, an Fe^{IV}=O porphyrin radical species (Compound I) and a $Fe^{IV}_2(m\text{-}O)_2$ species (Intermediate Q) have been found to be responsible oxidant for alkane hydroxylation and alkene epoxidation. Such the high valent iron-oxo species are inferred to involve in hydroxylation of aromatic compounds by mononuclear non-heme iron oxygenases, the reaction processes of which, however, still remains to be established. In order to gain insight into the active intermediates, we try to synthesize iron complexes with bulky schiff-base ligands as biomimetic models of mononuclear non-heme iron active sites. The active oxygen adduct of these complexes, which would be kinetically stabilized by their steric hindrance, might provide a basis for understanding the oxygenation by mononuclear iron sites.

VIII-D-6 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 is the terminal oxidase which reduces molecular oxygen (O_2) to water (H_2O), 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 in the catalytic core, and Cu_A and Heme-a as electron transfer sites in the backbone structure, respectively. 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 cytochrome c oxidase, 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. 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.

VIII-E 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 protoporphyrin IX (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.

VIII-E-1 Participation of Carboxylate Amino Acid Side Chain in Regiospecific Oxidation of Heme by Heme Oxygenase

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The regioselective oxidation of the α-meso position by HO is quite unique, in contrast to the non-enzymatic heme degradation that forms a mixture of four possible α, β, γ, δ-biliverdin isomers. The present study shows the first evidence of the formation of biliverdin isomers other than biliverdinIXα by HO mutants. The replacement of the highly conserved arginine 183(R183) of HO-1 with glutamic acid (E) or aspartic acid (D) forms biliverdinIXδ isomer along with normal biliverdinIXα. The absorption and EPR spectra and HO catalytic activity of R183E mutant are similar to those of the wild type heme-HO complex, indicating no significant change in the active site structure with mutation. To investigate the effects of the carboxylate functionalities introduced at the position 183, we prepared R183Q, R183N, R183A, R183T, and R183Y. The HO reactions of these mutants do not produce biliverdin isomers other than the normal biliverdinIXα. These results indicate that the carboxylate group introduced at position 183 is involved in the formation of δ-biliverdin isomer. The formation of δ-biliverdin isomer is expected to result in heme rotation through electronic repulsion between the carboxylate of E183 and heme propionate and/or change in distal side protein structure through a formation of new long-range hydrogen bond interaction network. All of the present results show the importance of the hydrogen bonding interaction between the arginine at position 183 and the carboxylates of the heme propionate group, as well as steric effect of the distal helix, for the α-regioselectivity.

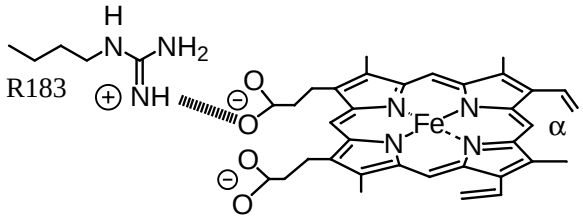

Figure 1. The hydrogen bonding interaction between R183 residue and the carboxylate of heme orients the heme to oxidize the α-meso position.