VIII-F 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-F-1 High-Spin (*meso*-Tetraalkylporphyrinato)-iron(III) Complexes As Studied by X-ray Crystallography, EPR, and Dynamic NMR Spectroscopies

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^{1}H-NMR spectra of a series of high-spin (*meso*-tetraalkylporphyrinato)iron(III) chlorides, [Fe(TRP)Cl] where R = Me, Et, Pr, or iPr, have been measured at various temperatures in CD_2Cl_2 solution. In the case of the Et, Pr, and iPr complexes, either the methyl or the methylene signal split into two signals with equal integral intensities at low temperature. In contrast, the Me complex did not show any splitting even at –100 °C. The results have been ascribed to the hindered rotation of the meso-alkyl groups about Cmeso-C bonds. The activation free energies for rotation have been determined as 8.0 (–72 °C), 8.5 (–60 °C), and 8.9 (–62 °C) kcal·mol^{-1} for the Et, Pr, and iPr complexes, respectively, at coalescence temperatures given in parentheses. The small activation free energy for rotation of the isopropyl groups observed in the present system is explained in terms of the nonplanarity of the porphyrin ring, which has been verified both by the X-ray crystallographic analysis and by the EPR spectrum taken in a frozen CH_2Cl_2-toluene solution. The success in observing the hindered rotation of less bulky primary alkyl groups such as ethyl and propyl groups at an easily accessible temperature range is attributed to the large difference in chemical shifts of the mutually exchanging protons, *ca*. 3500 Hz in the case of the Et complex, caused by the paramagnetism of the five-coordinated ferric porphyrin complexes.

VIII-F-2 Insensitivity of Vanadyl-Oxygen Bond Strengths to Radical Type ($^2A_{1u}$ *vs*. $^2A_{2u}$) in Vanadyl Porphyrin Cation Radicals

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Resonance Raman (RR) spectra are reported for vanadyl octaethylporphyrin, O=V(OEP), tetramesityltetramethylporphyrin, O=V(TMTMP), and tetramesitylporphyrin, O=V(TMP), and their corresponding π-cation radicals obtained by chemical and electrochemical oxidation. The behavior of the ν_2 RR porphyrin "marker band," which moves to higher frequency upon oxidation of the O=V(OEP) and O=V(TMTMP) and to lower frequency for O=V(TMP), shows that the resultant cation radicals have predominantly $^2A_{1u}$ and $^2A_{2u}$ ground states, respectively. In contrast to earlier work (K. A. Macor, R. S. Czernuszewicz and T. G. Spiro, *Inorg. Chem.* **29**, 1990 (1996)), it is demonstrated here that the shift of the ν(V=O) is insensitive to radical type, behavior which is in agreement with similar studies of the ferryl analogues (K. Czarnecki *et al.*, *J. Am. Chem. Soc.* **116**, 2929 and 4680 (1996)). It is suggested that the observed downshifts of the ν(V=O) previously reported for RR spectra of vanadyl porphyrin π-cation radicals, relative to their neutral parents, are most reasonably ascribed to trans oxo ligand coordination (most probably a water molecule) during low-temperature electrochemical oxidation of the neutral species.

VIII-F-3 Electron Configuration of Ferric Ions in Low-Spin (Dicyano)(meso-tetraarylporphyrinato)-iron(III) Complexes

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The electron configuration of a series of low-spin (dicyano){meso-tetrakis(2,4,6-trialkylphenyl)-porphyrinato}iron(III) complexes, $[Fe(R\text{-}TPP)(CN)_2]^-$ where R = Me, Et, or iPr, together with the parent $[Fe(TPP)(CN)_2]^-$, has been examined in dichloromethane-methanol solution by ^{1}H-NMR, ^{13}C-NMR, and EPR spectroscopies. While the ferric ion of $[Fe(TPP)(CN)_2]^-$ has shown a common $(d_{xy})^2(d_{xz},d_{yz})^3$ configuration, the ferric ions of the alkyl-substituted complexes $[Fe(R\text{-}TPP)(CN)_2]^-$ have exhibited the preference of a less common $(d_{xz},d_{yz})^4(d_{xy})^1$ configuration. Spectroscopic characteristics of the complexes in which ferric ions take the $(d_{xz},d_{yz})^4(d_{xy})^1$ configuration are (i) axial type EPR spectra, (ii) downfield shifted pyrrole and meta signals in ^{1}H-NMR spectra, and (iii) downfield shifted meso-carbon signals in ^{13}C-NMR spectra. Occurrence of the less common $(d_{xz},d_{yz})^4(d_{xy})^1$ configuration in $[Fe(R\text{-}TPP)(CN)_2]^-$ has been ascribed to the electronic interaction between iron(d) and cyanide(p*) orbitals. The interaction stabilizes the d orbitals and induces $(d_{xz},d_{yz})^4(d_{xy})^1$ configuration. Since the electron configuration of (dicyano){meso-tetrakis(2,6-dichloro-

phenyl)porphyrinato}iron(III), $[Fe(Cl\text{-}TPP)(CN)_2]^-$, which carries bulky electronegative chlorine atoms at the ortho positions, is presented as a common $(d_{xy})^2(d_{xz},d_{yz})^3$, the less common $(d_{xz},d_{yz})^4(d_{xy})^1$ configuration in $[Fe(R\text{-}TPP)(CN)_2]^-$ can be ascribed, at least partially, to the electron-donating ability of the meso-aryl groups.

VIII-F-4 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 $[O=Fe(TMP^{+\bullet})(2\text{-}MeIm)]^+$, which are stablized in dichloro-methane 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.

VIII-F-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 $Fe^{IV}_2(\mu\text{-}O)_2$ 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-F-6 ^{17}O-NMR Study of Oxygen Molecules Bound to Copper Ions of Mononucler and Dineucler Copper Complexes

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The activation of molecular oxygen by transition metals has fascinated chemists for decades. In particular copper-dioxygen complexes are suggested as key reaction intermediates in many enzymatic reactions. The differentiation in the function of these copper enzymes is attributed primarily to the coordination structure of the copper-dioxygen intermediate formed in the protein matrices, depending on the ligand donors, the geometry, and the coordination mode of the dioxygen. However, the correlation between these structural factors and the function/catalysis of the enzymes remains to be elucidated. To this end, there have been reported the structural and/or functional model complexes of copper-dioxygen adducts, such as μ-peroxo complex and μ-η_2,η_2 complex. The copper-bound dioxygen is not activated when it is end-on structure but activated via O-O bond cleavage when it is side-on structure. In order to investigate the relationships between electronic structure and reactivity of copper-dioxygen complex, we have examined ^{17}O-NMR spectroscopies of several copper-dioxygen complexes.

VIII-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 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-G-1 Molecular Oxygen Oxidizes the Porphyrin Ring of the Ferric α-Hydroxyheme in Heme Oxygenase in the Absence of Reducing Equivalent

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Heme oxygenase catalyzes the regiospecific oxidative degradation of iron protoporphyrin IX (heme) to biliverdin, CO and Fe, utilizing molecular oxygen and electrons donated from the NADPH-cytochrome P450 reductase. The catalytic conversion of heme proceeds through two known heme derivatives, α-hydroxyheme and verdoheme. In order to assess the requirement of reduction equivalents, we have prepared the α-hydroxyheme complex with rat heme oxygenase isoform-1 and examined its reactivity with molecular oxygen in the absence of added electrons. Upon reaction with oxygen, a minor portion of the α-hydroxyheme in heme oxygenase is converted to verdoheme with the majority altered to a species which exhibits an optical absorption spectrum with a broad Soret band. The major species, which is EPR-silent, can be converted to the original α-hydroxyheme by addition of sodium dithionite. We have also found that oxidation of the α-hydroxyheme-heme oxygenase complex by ferricyanide or iridium chloride yields a species which exhibits an optical absorption spectrum and reactivity similar to those of the main product of the oxygen reaction. We infer that the oxygen reaction with the ferric α-hydroxyheme-heme oxygenase complex forms a ferric-porphyrin cation radical. We conclude, inconsistent to a previous report (Y. Liu, P. Moenne-Loccoz, T. M. Loehr and P. R. Ortiz de Montellano, *J. Biol. Chem.* **272**, 6909 (1997)), that in the absence of reducing agents, the oxygen molecule functions mainly as an oxidant for the porphyrin ring and has no role in the oxygenation of α-hydroxyheme. This result corroborates our previous conclusion that the catalytic conversion of α-hydroxyheme to verdoheme by heme oxygenase requires one reducing equivalent along with molecular oxygen.

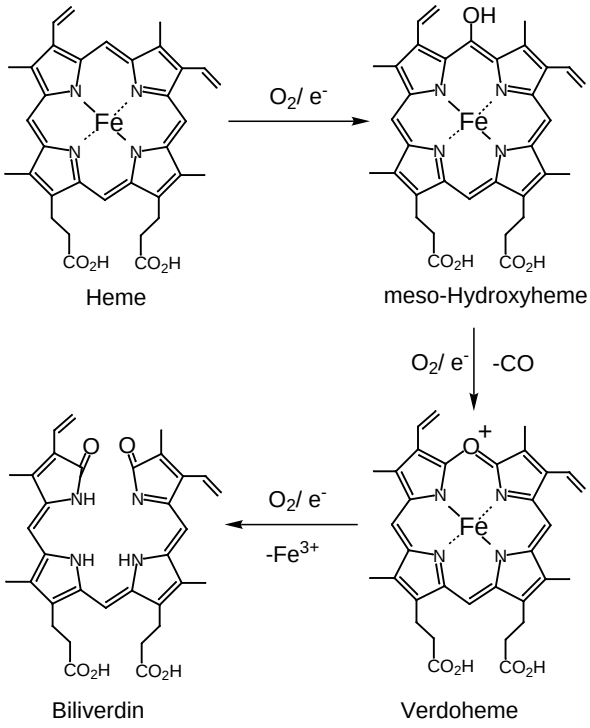

Figure 1. Reaction intermediates in the heme oxygenase catalyzed oxidation of heme to biliverdinIXα.