X-C Reaction Mechanism of Metalloenzymes Related to Oxygen Activation

Oxygen is quite important molecule for most organisms. Oxygen is utilized for various physiological functions such as ATP synthesis, defense mechanism, oxidation reactions, and signal transduction. These diverse functions are realized by many metalloenzymes. In this project, we are studying molecular mechanisms of these metalloenzymes.

X-C-1 Oxidizing Intermediates from the Sterically Hindered Salen Iron Complexes Related to the Oxygen Activation by Nonheme Iron Enzymes

KURAHASHI, Takuya; KOBAYASHI, Yoshio[1]; NAGATOMO, Shigenori; TOSHA, Takehiko; KITAGAWA, Teizo; FUJII, Hiroshi
([1]RIKEN)

[*Inorg. Chem*. **44**, 8156–8166 (2005)]

Oxidizing intermediates are generated from nonheme iron(III) complexes to investigate the electronic structure and the reactivity, in comparison with the oxoiron(IV) porphyrin π-cation radical (compound I) as a heme enzyme model. Sterically hindered salen iron complexes, bearing a fifth ligand Cl (**1**), OH_2 (**2**), OEt (**3**) and OH (**4**), are oxidized both electrochemically and chemically. Stepwise one-electron oxidation of **1** and **2** generates iron(III)–mono- and diphenoxyl radicals, as revealed by detailed spectroscopic investigations, including UV-Vis, EPR, Mössbauer, resonance Raman, and ESIMS spectroscopies. In contrast to the oxoiron(IV) formation from the hydroxoiron(III) porphyrin upon one-electron oxidation, the hydroxo complex **4** does not generate oxoiron(IV) species. Reaction of **2** with *m*CPBA also results in the formation of the iron(III)–phenoxyl radical. One-electron oxidation of **3** leads to oxidative degradation of the fifth EtO ligand to liberate acetaldehyde even at 203 K. The iron(III)–phenoxyl radical shows high reactivity for alcoxide on iron(III), but exhibits virtually no reactivity for alcohols including even benzyl alcohol without a base to remove an alcohol proton. The present study explains unique properties of mononuclear nonheme enzymes with Tyr residues, and also a poor epoxidation activity of Fe salen compared to Mn and Cr salens.

X-C-2 A Trigonal-Bipyramidal Geometry Induced by an External Water Ligand in a Sterically Hindered Iron Salen Complex, Related to the Active Site of Protocatechuate 3,4-Dioxygenase

KURAHASHI, Takuya; ODA, Kenji[1]; SUGIMOTO, Manabu[2]; OGURA, Takashi[1]; FUJII, Hiroshi
([1]Univ. Hyogo; [2]Kumamoto Univ.)

[*Inorg. Chem.* in press]

A unique distorted trigonal-bipyramidal geometry observed for the nonheme iron center in protocatechuate 3,4-dioxygenase (3,4-PCD) was carefully examined utilizing a sterically hindered iron salen complex, which well reproduces the endogenous His_2Tyr_2 donor set with water as an external ligand. X-ray crystal structures of a series of iron model complexes containing bis(3,5-dimesitylsalicylidene)-1,2-dimesitylethylenediamine indicate that a distorted trigonal-bipyramidal geometry is achieved upon binding of water as an external ligand. The extent of a structural change of the iron center from a preferred square-pyramidal to a distorted trigonal-bipyramidal geometry varies with the external ligand that is bound in the order Cl << EtO < H_2O, which is consistent with the spectrochemical series. The distortion in the model system is not due to steric repulsions, but electronic interactions between the external ligand and the iron center, as evidenced from the X-ray crystal structures of another series of iron model complexes with a less-hindered bis(3-xylylsalicylidene)-1,2-dimesitylethylenediamine ligand, as well as by DFT calculations. Further spectroscopic investigations indicate that a unique distorted trigonal-bipyramidal geometry is indeed maintained even in solution. The present model study provides a new viewpoint that a unique distorted trigonal-bipyramidal iron site might not be preorganized by a 3,4-PCD protein, but could be electronically induced upon the binding of an external hydroxide ligand to the iron(III) center. The structural change induced by the external water ligand is also discussed in relation to the reaction mechanism of 3,4-PCD.

X-C-3 O_2- and H_2O_2-Dependent Verdoheme Degradation by Heme Oxygenase: Reaction Mechanisms and Potential Physiological Roles of the Dual Pathway Degradation

MATSUI, Toshitaka[1]; NAKAJIMA, Aya[1]; FUJII, Hiroshi; YOSHIDA, Tadashi[2]; IKEDA-SAITO, Masao[1]
([1]Tohoku Univ.; [2]Yamagata Univ.)

[*J. Biol. Chem*. **280**, 36833–36840 (2005)]

Heme oxygenase (HO) catalyzes catabolism of heme to biliverdin, CO and a free iron through three successive oxygenation steps. The third oxygenation, oxidative degradation of verdoheme to biliverdin, has been the least understood step in spite of its importance to regulate the HO activity. We have thoroughly examined degradation of a synthetic verdoheme IXα complexed with rat HO-1. Our major findings include: (1) HO degrades verdoheme through a dual pathway using either O_2 or H_2O_2; (2) the newly found H_2O_2 pathway is approximately 40-fold faster than the O_2-dependent degradation; (3) both reactions are initiated by the binding of O_2 or H_2O_2 to allow the first direct obser-

vation of degradation intermediates of verdoheme; and (4) Asp^{140} in HO-1 is critical for the verdoheme degradation regardless of the oxygen source. On the basis of these findings, we propose that the HO enzyme activates O_2 and H_2O_2 on the verdoheme iron with the aid of a nearby water molecule linked with Asp^{140}. These mechanisms are similar to a well-established mechanism of the first oxygenation, *meso*-hydroxylation of heme, and thus, HO can utilize a common architecture to promote the first and third oxygenation steps of the heme catabolism. We also point out a possible involvement of the H_2O_2-dependent verdoheme degradation *in vivo*, and propose potential roles of the dual pathway reaction of HO against oxidative stress.

X-C-4 ^{13}C and ^{15}N NMR Studies of Iron-Bound Cyanides of Heme Proteins and Related Model Complexes: Sensitive Probe for Detecting Hydrogen Bonding Interactions at the Proximal and Distal Sides

FUJII, Hiroshi; YOSHIDA, Tadashi[1]
([1]Yamagata Univ.)

[*Inorg. Chem.* **45**, 6816–6827 (2006)]

Studies of the ^{13}C and ^{15}N NMR paramagnetic shifts of the iron bound cyanides in the ferric cyanide forms of various heme proteins containing the proximal histidine and related model complexes are reported. The paramagnetic shifts of the ^{13}C and ^{15}N NMR signals of the iron bound cyanide are not significantly affected by the substitution of the porphyrin side chains. On the other hand, the paramagnetic shifts of both ^{13}C and ^{15}N NMR signals decrease with an increase in the donor effect of the proximal ligand and the ^{13}C NMR signal is more sensitive to a modification of the donor effect of the proximal ligand than the ^{15}N NMR signal. With the tilt of the iron–imidazole bond, the paramagnetic shift of the ^{13}C NMR signal increases whereas that of the ^{15}N NMR signal decreases. The hydrogen bonding interaction of the iron bound cyanide with a solvent decreases the paramagnetic shift of both ^{13}C and ^{15}N NMR signals, and the effect is more pronounced for the ^{15}N NMR signal. Data on the ^{13}C and ^{15}N NMR signals of iron bound cyanide for various heme proteins are also reported and analyzed in detail. Substantial differences in the ^{13}C and ^{15}N NMR shifts for the heme proteins can be explained based on the results for the model complexes and structures around the heme in the heme proteins. The findings herein show that the paramagnetic shift of the ^{13}C NMR signal of the iron bound cyanide is a good probe to estimate the donor effect of the proximal imidazole and that the ratio of $^{15}N/^{13}C$ NMR shifts allows estimating the hydrogen bonding interaction on the distal side.

X-C-5 Roles of the Heme Distal Residues of FixL in O_2 Sensing: A Single Convergent Structure of the Heme Moiety Is Relevant to the Downregulation of Kinase Activity

TANAKA, Atsunari[1]; NAKAMURA, Hiro[1]; SHIRO, Yoshitsugu[1]; FUJII, Hiroshi
([1]RIKEN)

[*Biochemistry* **45**, 2515–2523 (2006)]

FixL is a heme-based O_2 sensor, in which the autophosphorylation is regulated by the binding

of exogenous ligands such as O_2 and CN^-. In this study, mutants of the heme distal Arg200, Arg208, Ile209, Ile210, and Arg214 residues of *Sm*FixL were characterized biochemically and physicochemically, because it has been suggested that they are significant residues in ligand-linked kinase regulation. Measurements of the autoxidation rate, affinities, and kinetics of ligand binding revealed that all of the above residues are involved in stabilization of the O_2-heme complex of FixL. However, Arg214 was found to be the only residue that is directly relevant to the ligand-dependent regulation of kinase activity. Although the wild type and R214K and R214Q mutants exhibited normal kinase regulation, R214A, R214M, R214H, and R214Y did not. ^{13}C and ^{15}N NMR analyses for $^{13}C^{15}N^-$ bound to the truncated heme domains of the Arg214 mutants indicated that, in the wild type and the foregoing two mutants, the heme moiety is present in a single conformation, but in the latter four, the conformations fluctuate possibly because of the lack of an interaction between the iron-bound ligand and residue 214. It is likely that such a rigid conformation of the ligand-bound form is important for the downregulation of histidine kinase activity. Furthermore, a comparison of the NMR data between the wild type and R214K and R214Q mutants suggests that a strong electrostatic interaction between residue 214 and the iron-bound ligand is not necessarily required for the single convergent structure and eventually for the downregulation of FixL.

X-C-6 ^{17}O NMR Study of Oxo Metalloporphyrin Complexes: Correlation with Electronic Structure of M=O Moiety

FUJII, Hiroshi; KURAHASHI, Takuya; TOSHA, Takehiko; YOSHIMURA, Tetsuhiko[1]; KITAGAWA, Teizo
([1]Yamagata Promotional Org. Industrial Tech.)

[*J. Inorg. Biochem.* **100**, 533–541 (2006)]

^{17}O NMR spectroscopy of oxo ligand of oxo metalloporphyrin can be considered as an excellent means to derive information about structure, electronic state, and reactivity of the metal bound oxo ligand. To show the utility of ^{17}O NMR spectroscopy of oxo ligand of oxo metalloporphyrin, ^{17}O-NMR spectra of oxo ligands of dioxo ruthenium(VI), oxo chromium(IV), and oxo titanium(IV) porphyrins are measured. For all oxo metalloporphyrins, well-resolved ^{17}O NMR signals are detected in far high frequency region. The ^{17}O NMR signal of the metal bound oxo ligand shifts high frequency in order of Ru(VI) < Ti(IV) < Cr(IV), thus the ^{17}O NMR chemical shift does not directly correlate with the oxo-transfer reactivity, Ti(IV) < Cr(IV) < Ru(VI). On the other hand, the ^{17}O NMR shift of oxo ligand correlates with the bond strength of metal–oxo bond. This suggests that the ^{17}O NMR signal of metal bound oxo ligand is a sensitive

probe to study the nature of metal–oxo bond in oxo metalloporphyrin. The effect of the electron-withdrawing meso-substituent on the ^{17}O NMR shift of the oxo ligand is also investigated. With increase in the electron-withdrawing effect of the meso-subsituent, the ^{17}O NMR signal of the oxo ligand of oxo chromium(IV) porphyrin shifts high frequency while that of dioxo ruthenium(VI) porphyrin hardly change resonance position. The changes in metal–oxo bonds induced by the electron-withdrawing meso-substituent are discussed on the basis of the ^{17}O NMR shifts, the strengths of the metal–oxo bonds, and the oxo-transfer reaction rates.

X-D Reaction Mechanism of Metalloenzymes Related to Global Nitrogen Cycle

For all organisms, organic nitrogen and ammonia are required as a constituent part of the cell. In order to keep the environment of the earth constant, the organic nitrogen, fixed nitrogen, must be completely reconverted into dinitrogen gas. The reverse process of the nitrogen fixing is called denitrification process. In this process, nitrate or nitrite ion is reduced to nitrogen gas *via* nitric oxide and nitrous oxide by many metalloenzymes, nitrate reducatse, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. In this project, we are studying the molecular mechanism of these metalloenzymes relating to the denitrification process.

X-D-1 Kinetic Isotope Effects on the Rate-Limiting Step of Heme Oxygenase Catalysis Indicate Concerted Proton Transfer/Heme Hydroxylation

KUJIME, Masato; FUJII, Hiroshi

[*Angew. Chem., Int. Ed*. **45**, 1089–1092 (2006)]

Reduction of nitrite (NO_2^-) to gaseous nitric oxide (NO) is one of key processes in the global nitrogen cycle and carried out by bacterial copper-containing nitrite reductases (NiR). The enzymes contain two copper ion centers: the type 1 copper site for electron transfer and the type 2 copper site for the catalytic nitrite reduction. Crystallographic and spectroscopic studies of Nir have proposed the mechanism of the nitrite reduction at the type 2 copper site. The enzyme reaction is initiated by the binding of the nitrite to the reduced form of the type 2 copper site to yield a copper(I) nitrite complex. Subsequently, the copper bound nitrite is reduced to NO and water with intramolecular one electron transfer from the type 2 copper(I) ion and two protons from a conserved aspartic acid placed near the type 2 site. During the course of synthetic study of the copper(I) nitrite complexes, we found that the rapid mixing of copper(I) nitrite complex with trifluoroacetic acid (TFA) with stopped flow at low temperature allows to detect new reaction intermediates in the reduction process. Here, we report detection and characterization of new reaction intermediates in the nitrite reduction. This study shows that two protons required for the reaction are not provided to the copper bound nitrite simultaneously but stepwise and that the intramolecular electron transfer from the copper(I) ion to the copper bound nitrite occurs in the second protonation step.

X-D-2 ^{63}Cu NMR Spectroscopy of Copper(I) Complexes with Various Tridentate Ligands: CO as a Useful ^{63}Cu NMR Probe for Sharpening ^{63}Cu NMR Signals and Analyzing the Electronic Donor Effect of a Ligand

KUJIME, Masato; KURAHASHI, Takuya; TOMURA, Masaaki; FUJII, Hiroshi

[*Inorg. Chem*. in press]

^{63}Cu NMR spectroscopic studies of copper(I) complexes with various N-donor tridentate ligands are reported. As has been previously reported for most copper(I) complexes, ^{63}Cu NMR signals, when acetonitrile is coordinated to copper(I) complexes of these tridentate ligands, are extremely broad or undetectable. However, when CO is bound the above tridentate copper(I) complexes, the ^{63}Cu NMR signals become much sharper and show a large downfield shift, compared to those for the corresponding acetonitrile complexes. Temperature dependence of ^{63}Cu NMR signals for these copper(I) complexes show that a quadrupole relaxation process is much more significant to their ^{63}Cu NMR line widths than a ligand exchange process. Therefore, an electronic effect of the copper bound CO makes the ^{63}Cu NMR signal sharp and easily detected. The large downfield shift for the copper(I) carbonyl complex can be explained by a paramagnetic shielding effect induced by the copper bound CO, which amplifies small structural and electronic changes that occur around the copper ion to be easily detected in their ^{63}Cu NMR shifts. This is evidenced by the correlation between the ^{63}Cu NMR shifts for the copper(I) carbonyl complexes and their ν(C≡O) values. Furthermore, the ^{63}Cu NMR shifts for copper(I) carbonyl complexes with imino type tridentate ligands show a different correlation line with those for amino type tridentate ligands. On the other hand, ^{13}C NMR shifts for the copper bound ^{13}CO for these copper(I) carbonyl complexes do not correlate with the ν(C≡O) values. The X-ray crystal structures of

these copper(I) carbonyl complexes do not show any evidence of a significant structural change around the Cu–CO moiety. The findings herein show that CO has great potential as a probe in ^{63}Cu NMR spectroscopic studies for characterizing the nature of the environment around copper ions in copper complexes.