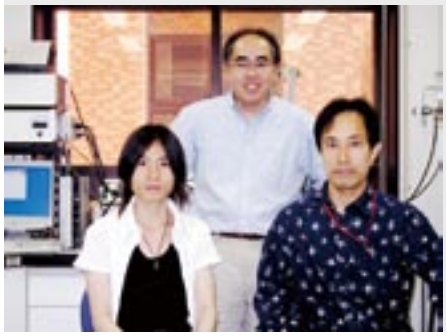
Structure-Function Relationship of Metalloproteins

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



FUJII, Hiroshi	Associate Professor
KURAHASHI, Takuya	Assistant Professor
KUJIME, Masato	Post-Doctoral Fellow*
TAKAHASHI, Akihiro	Graduate Student
TANIZAWA, Misako	Secretary

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 electronic structures of the metal active sites and reactivity of metalloproteins.

1. 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[1)]

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 (Figure 1). 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.

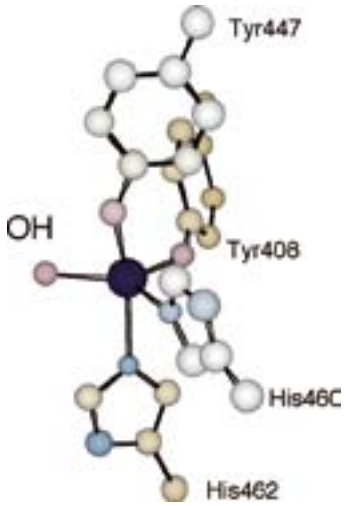


Figure 1. Active site structure of Protocatechuate 3,4-dioxygenase.

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[2)]

^{63}Cu NMR spectroscopic studies of copper(I) complexes with various N-donor tridentate ligands, shown in Figure 1, are reported. As has been previously reported for most copper(I) complexes, ^{63}Cu NMR signals, when acetonitrile is coordi-

nated 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.

3. Activation Parameters for Cyclohexene Oxygenation by Oxoiron(IV) Porphyrin π-Cation Radical Complex: Entropy Control of Allylic Hydroxylation Reaction[3)]

Cytochromes P450 (P450) are very versatile catalysts, which activate molecular oxygen and catalyze hydrocarbon hydroxylation or alkene epoxidation with high stereoselectivity. Reaction of P450 with cyclohexene yields a mixture of two major products: cyclohexene oxide (an epoxidation product) and 2-cyclohexen-1-ol (an allylic hydroxylation product). Interestingly, the ratio of epoxidation to allylic hydroxylation products, *i.e.*, the chemoselectivity, is changed by P450 isozymes and by mutation of a single amino acid near the proximal or distal side. In addition, P450 model studies using synthetic iron porphyrin complexes showed that the chemoselectivity depends on various other factors, such as the nature of the porphyrin and axial ligands, solvents, and reaction temperature. While these enzymatic and model studies suggest that chemoselectivity is dependent on the electronic structure of the reactive intermediate, oxoiron(IV) porphyrin π-cation radical species (compound I), it is not clear how compound I controls chemoselectivity. Recently, the reaction mechanism and chemoselectivity of P450 have been studied by theoretical calculations based on density functional theory (DFT). In DFT studies, reaction mechanism and chemoselectivity are predicted from calculated activation energy, E_a, for epoxidation and allylic hydroxylation reactions of oxoiron(IV) porphyrin π-cation radical species. These studies are based on the assumption that the contribution of entropy of activation, $\Delta S^{\ddagger}$, is much smaller than that of enthalpy of activation, $\Delta H^{\ddagger}$. However, the validity of this assumption remains unproven because the activation parameters for epoxidation and allylic hydroxylation reactions of compound I species are yet to be determined. To better understand epoxidation and allylic hydroxylation reactions, the present study ascertained the activation parameters for epoxidation and allylic hydroxylation reactions of cyclohexene with compound I species, $Fe^{IV}O$ $(TMP)^{+\bullet}Cl$ (**1**), (Figure 2). This study demonstrated that epoxidation is an enthalpy-controlled reaction, while allylic hydroxylation is an entropy-controlled reaction. The large contribution of the entropy term, $-T\Delta S^{\ddagger}$, to the free energy of activation, $\Delta G^{\ddagger}$, indicated that $\Delta G^{\ddagger}$, rather than E_a, should be used to predict reaction mechanisms and chemoselectivity.

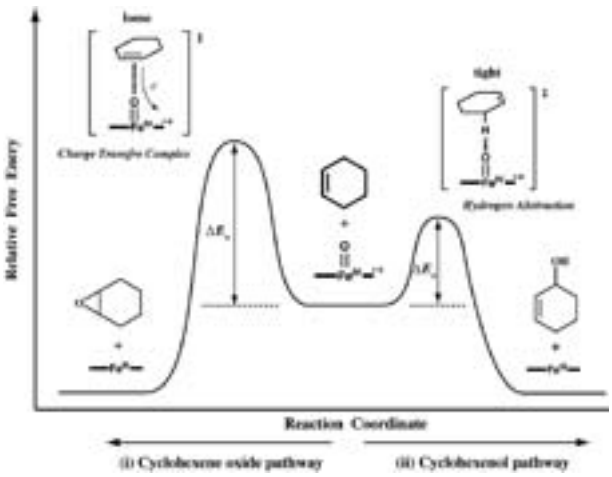


Figure 2. A cartoon of the reaction coordinates for allylic hydroxylation and epoxidation of cyclohexene by oxoiron(IV) porphyrin π-cation radical complex.

References

1) T. Kurahashi, K. Oda, M. Sugimoto, T. Ogura and H. Fujii, *Inorg. Chem.* **45**, 7709–7721 (2006).
2) M. Kujime, T. Kurahashi, M. Tomura and H. Fujii, *Inorg. Chem.* **46**, 541–551 (2007).
3) A. Takahashi, T. Kurahashi and H. Fujii, *Inorg. Chem.* **46**, 6227–6229 (2007).

Award

TAKAHASHI, Akihiro; Award for Oral Presentation by Graduate Student in Annual Meeting of Chemical Society of Japan.

* Present Address; Advanced Industrial Science and Technology, Tsukuba 305-8565