RESEARCH ACTIVITIES VII
Coordination Chemistry Laboratories

Professor Yasuhiro Uozumi and Associate Professor Hiroyuki Kawaguchi joined to laboratories of Synthetic Coordination Chemistry and Functional Coordination Chemistry. Prof. Isao Nishida, Prof. Yasutaka Tanaka took the position of Synthetic Coordination Chemistry from April 1998. Prof. Hiromu Sakurai (Kyoto Pharmacy Univ.) and Assoc. Prof. Yasushi Mizobe (Univ. Tokyo) finished their term as Adjunct Prof. in March 2000 in the Laboratory of Coordination Bond. Their effort during their term is gratefully appreciated. Prof. Takuzo Aida (Univ. Tokyo) and Assoc. Prof. Itaru Hamachi (Kyushu Univ.) continue the position of the laboratory of Coordination Bond.

VII-A New Insight into Mechanism of Oxygen Activation in Biological Oxygenases

One of the remaining frontiers in organic chemistry is the direct functionalization of saturated hydrocarbons. The catalytic cycle that oxidizes a hydrocarbon R–H to an alcohol R–OH employing cytochrome P-450 and methane monooxygenase is a well-established process, however no reasonable mechanism for oxygen activation and for formation of the R-OH is available at present. Recently the present author has proposed a new idea that elucidates many biological oxygenation reactions including monooxygenases and dioxygenases comprehensively. In this new concept, the importance of electrophilic nature of a metal-peroxide adduct and the role of the substrate as an electron donor to the peroxide adduct were emphasized (Y. Nishida, *Trends Inorg. Chem.* **5**, 89 (1998)). This idea has been supported by many experimental facts, especially by the work of Sligar *et al.* (*Science* **287**, 1615 (2000)). We are now continuing the study on the reactivity of the metal-peroxide adducts in order to ascertain that my idea is applicable to other reactions, such as degradation of DNA and proteins by the metal-peroxide adducts.

VII-A-1 Interaction between a Copper(II) Compound and Protein Investigated in terms of the Capillary Electrophoresis Method

NISHINO, Satoshi; ISHIKAWA, Yoshihiro; NISHIDA, Yuzo

[*Inorg. Chem. Commun.* **2**, 438 (1999)]

Recently there have been a number of reports on peptides which aggregate in ways that may be relevant to their biological activity. The formation of amyloid deposits in Alzheimer's disease and the conversion of PrP^{C} (the normal cellular prion protein) into PrP^{Sc} and its truncated form PrP 27–30, the abnormal disease-causing isoform) in certain human and animal neurodegenerative diseases are typical examples. Very recently we have postulated that a copper(II)-peroxide adduct plays an important role in the formation of PrP^{Sc} protein and its truncated form PrP 27-30. However the mechanism of conversion from PrP^{C} into PrP^{Sc} is unknown at present. In order to elucidate the above problems it seems necessary to obtain detailed information on the interaction between the metal chelaltes and protein, but at present there are few suitable ways to study the interaction between the protein and a small metal chelate. In this paper, we have shown that the capillary electrophoresis method is very useful for studying the interaction in question.

VII-A-2 Contribution of a Metal-Peroxide Adduct to Neurodegeneration is due to its Oxidase Activity

NISHIDA, Yuzo; NISHINO, Satoshi

[*J. Bioscience* **54C**, 1107 (1999)]

Many hypothesis have been developed to explain aging and age-related neurodegenerative diseases, one of he most compelling is the role of oxidative stress to induce changes in protease activity in brains of patients of Alzheimer's disease and prion diseases. At the moment, however, there is no clear answer how protein degradation may be achieved in the brain. We have observed that several metal compounds can degrade proteins in the presence of hydrogen peroxide, and elucidated the reaction scheme based on the new theoretical point for the reactivity of a metal-peroxide adduct with η^1-coordination mode. In this article we have pointed out the importance of a copper(II)-peroxide adduct to promote neurodegenerative diseases such as prion disease and amyotrophic lateral sclerosis through its oxidative protease activity.

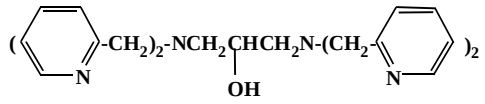
VII-A-3 DNA Promotes the Activation of Oxygen Molecule by Binuclear Cobalt(II) Compounds

NISHIDA, Yuzo; NISHINO, Satoshi; GUO, Li Li; KUNITA, Mami; MATSUSHIMA, Hideaki; TOKII, Tadashi

[*Inorg. Chem. Commun.* **2**, 609 (1999)]

During the past decades there has been an explosion in the research effort directed towards the isolation and evaluation of naturally occurring DNA cleaving agents and towards the design and synthesis of model compounds that can specifically recognize and cut DNA. The potential scope of the utility of these compounds ranges from the creation of synthetic restriction enzymes for use of biologists to the development of chemotherapeutic agents that may be effective against a variety of neoplastic diseases. The

bleomycins (BLM) are family of glycopeptide-derived antibiotics, discovered by Umezawa and co-workers, which have been used clinically against certain malignant lymphomas and squamous cell carcinomas. The therapeutic activity of BLM is generally believed to correlate with the ability of an "active-BLM," which is derived from either Fe(II)-BLM/O_2 or Fe(III)-BLM/H_2O_2 system, to bind to and degrade DNA. This means that Fe-BLM is a dangerous species for the human beings, because it always contains an active oxygen species, although the active bleomycin is quickly bleached when this drug is activated outside of this target(DNA). The most desirable agent for clinical use, should be that the agent cleaves DNA oxidatively through production of an active oxygen species only when it reacts with DNA. In this report we have showed that some binuclear cobalt(II) compounds with H(HPTP)(see below) belong to this type of compounds.



H(HPTP)

VII-A-4 Structure and Function of "Free Iron Ion" in Biological System and Their Model Compounds

NISHIDA, Yuzo

[*Recent Res. Dev. Pure Appl. Chem.* **3**, 103 (1999)]

Iron is as essential participant in many human metabolic processes, but recent studies on neurodegenerative diseases have revealed that free ion, *i.e.*, excess iron ion in the cell, is potentially dangerous, and abnormalities in rain iron metabolism have been described for several neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. In this study we have prepared several model compounds for the "free iron(II) ion" in the cell, and discussed origin of the free iron ion formation, and the mechanism of oxygen activation, the cell damage, and cancer process by the free iron ion in biology. We have pointed out that hydrogen peroxide and free iron(III) ion which is captured by a chelate containing peptide-group should be a serious origin for the iron ion toxicity in cells.

VII-A-5 New Insight into Oxidative DNA Cleavage Reaction Catalyzed by Metal Chelates

NISHIDA, Yuzo

[*Recent Res. Dev. Pure Appl. Chem.* **3**, 123 (1999)]

Mechanism of oxidative DNA cleavage reactions catalyzed by a metal compound was re-considered based on the new concept on the chemical reactivity of the metal-peroxide adduct. The fact that chemically inert Co(III)-bleomycin-OOH mediate DNA strand scission only when irradiated at 366 nm light, and the recent results on the steric interaction between the Co(III)-bleomycin-OOH and oligomer investigated by NMR technique has lead to the conclusion that photoirradiation at 366 nm on the Co(III)-bleomycin-OOH induces d-d transition (from t_{2g} orbital to d_{z^2}-orbital) and charge transfer transition (from the ligand orbital to d_{z^2}-orbital), leading to facile O-O heterolysis of the peroxide ion; after this terminal oxygen atom is transferred directly into C(4'-position)-H bond of the sugar moiety, to give the corresponding hydroxylated derivative, leading to strand scission. The results obtained from the model compounds including Fe(III) and Cu(II) all support the above conclusion, and new mechanism for DNA cleavage reaction, which excludes the possible formation of 4'-carbon radical as an intermediate, was proposed for DNA cleavage by the Fe(III)-bleomycin.

VII-A-6 Cleavage of C–N bond of Peptide Group by Copper(II)-peroxide Adduct with η^1-Coordination Mode

NISHINO, Satoshi; KUNITA, Mami; KANI, Yoshiyuki; OHBA, Shigeru; MATSUSHIMA, Hideaki; TOKII, Tadashi; NISHIDA, Yuzo

[*Inorg. Chem. Commun.* **3**, 145 (2000)]

The bioactivation of many peptide hormones and neuropeptides involves oxidative cleavage of carboxy-terminal glycine-extended precursors. The process is catalyzed by the enzyme peptidylglycine α-amidating monooxygenase (PAM), which comprises two subunits. One of these, requires copper ion, ascorbate and molecular oxygen, and facilitates α-hydroxylation of glycine residues. A range of chemical models of PAM has been developed, however the detailed reaction mechanism of cleavage of C-N bond is not clear at present.

Very recently we have reported that some copper(II) compounds exhibit high activity for degrading albumin in the presence of hydrogen peroxide, and postulated that a copper(II)-peroxide adduct may play an important role in the formation of PrP 27-30 and PrP^{Sc}, which are believed to be an abnormal disease-causing isoform of prion protein, and also in conformational change in mutated SOD enzyme observed for ALS patients. In this study we have obtained a clear evidence to support that a copper(II)-peroxide adduct with η^1-Coordination mode can cleave the C-N bond of peptide group and hydroxylate the alkyl group nearby; this may give a new idea to elucidate the reaction mechanism of PAM enzyme.

VII-A-7 Important role of Proton in Activation of Oxygen Molecule in Heme-Containing Oxygenases

NISHIDA, Yuzo

[*Inorg. Chem. Commun.* **3**, 310 (2000)]

Peroxoiron(III) complexes are increasingly being considered as potential intermediates in oxidation reactions catalyzed by both non-heme and heme-

centers. In cytochrome P-450 and heme-oxygenase, a hydroperoxide adduct of Fe(III) with η^1-coordination mode has been considered to be an important intermediate but detailed electronic property and reactivity of the peroxide adduct are less known at present. In recent years, Density Functional Theory (DFT) has emerged as an accurate alternative first-principles approach to quantum mechanical molecular investigations. DFT currently accounts for approximately 90% of all quantum chemical calculations being performed, not only because of its proven chemical accuracy, but also of its relatively cheap computational expense. In this study we have investigated the electronic property of the peroxide adduct of the heme compounds in terms of DFT.

DFT (density-functional theory) calculations have revealed that the position of proton of a hydroperoxide adduct of Fe(III)-porphyrin compound influences greatly the electron density on the oxygen atoms of the peroxide ion, suggesting that the position of proton in the hydroperoxo-iron(III) compound plays an important role in controlling the electronic interaction between the hydroperoxide adduct and porphyrin system (OOH-π interaction). These are very useful to consider the reaction mechanism of the both cytochrome P450 and heme-oxygenase.