RESEARCH ACTIVITIES V
Department of Applied Molecular Science

V-A Molecular Design and Functions of Photoactive and Spin-Active Supramolecular Assemblies

To achieve photosynthesis with a totally artificial system is a supreme challenge in science and a dream of molecular scientist. In nature, plants and photosynthetic bacteria depend on photosynthesis utilizing elaborate chromophore arrays to trap solar energy, followed by an efficient energy transfer to the reaction center. Although there have been many efforts to design molecular systems for light-harvesting, they usually surfer from inadequate energy transfer efficiency. Synthetic macromolecules have attracted attention as potential photosynthetic antennae, since they can be incorporated and organized. However, examples thus far reported are generally derived from linear-chain polymers, which, unlike biological macromolecules, can adopt ill-defined morphologies, many of which lead to complicated photochemical events associated with intra- and interchain interactions. Moreover, broad molecular weight distribution and uncontrolled structures inherent in linear chain synthetic polymers, make it difficult to develop meaningful correlation between their structures and photochemical functions.

In this project, we are focusing on development of novel nanomaterials for the exploitation of new functions and properties through molecular design and programmed self-assembly. Especially, creation of novel nanomaterials exhibiting high capability of controlling photoinduced energy transfer and photoinduced electron transfer is one of important missions.

In relation to the above project, we are intended to exploit spatially well-defined dendritic macromolecules for highly controlled arrays of supramolecular and macromolecular metallo–complexes with the goal of developing unique functions that are impossible with small molecules. In detail, we aim for demonstration of principles for molecular design of spin-active nanomaterials, realization of photo-induced spin transition and control of spin state in confined space, and creation of functional nanomaterials for future-generation spin devices.

V-A-1 Molecular Design of Light-Harvesting Antennae

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[*Dendritic Polymers* (in Japanese), NTS, p. 2–21 (2005)]

In the present chapter, we highlight our recent efforts to construct several new bioinspired dendrimers and their self-organization by focusing attention on structure–function relationships.

V-B Bioinorganic Chemistry and Structural Biology of Heme Proteins

One of research activities of my group is directed toward developing a rigorous, quantitative understanding of the biochemical function of heme proteins such as oxygenases, peroxidases and oxidases by characterization of their structural and functional properties. We use different experimental strategies including protein engineering, spectroscopic characterization of the molecular structure of the active centers, measurements of dynamics of substrates and inhibitor binding, and X-ray crystallography.

My current heme protein projects include (1) elucidation of the catalytic mechanism of heme oxygenase, one of the essential components of the heme catabolism and biosynthesis of carbon monoxide, a versatile physiological messenger molecule, (2) elucidation of the mechanism of controlling reactivity of hemoglobin and myoglobin, and (3) determination of heme sensing mechanism of Bach1, a heme-dependent transcription factor which regulates heme oxygenase gene expression. Effective clues to delineate the detailed active site structure have been obtained by X-ray crystallography, resonance Raman and magnetic resonance studies. The synergy of site-directed mutagenesis, structural biology, and spectroscopic techniques has revealed the specific roles of amino acids located in the active centers of heme proteins. Ligands and substrates binding measurements complement the structural data for our understanding functional properties displayed by heme proteins at the molecular level.

V-B-1 Proton Transfer at Helium Temperatures during Dioxygen Activation by Heme Monooxygenases

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[*J. Am. Chem. Soc*. **126**, 15960–15961 (2004)]

Heme oxygenase (HO) catalyzes the O_2 and NADPH/cytochrome P450 reductase-dependent conversion of heme to biliverdin, free iron ion, and CO through a process in which the heme participates both as dioxygen-activating prosthetic group and substrate. In the first measurement of enzymatic proton transfer at liquid helium temperatures, we examine protonation of the peroxo-ferriheme state HO produced by *in situ* radiolytic cryoreduction of oxy-HO in H_2O and D_2O solvents at *ca.* 4 K and above, and compare these findings with analogous measurements for oxy-P450cam and for oxy-Mb. Proton transfer in HO occurs at helium temperatures in both solvents; it occurs in P450cam at ~50 K and higher; in Mb it does not occur until $T > 170$ K. For Mb, this transfer at 180 K is biphasic, and the majority phase shows a solvent kinetic isotope effect of 3.8. We discuss these results in the context of the picture of environmentally coupled tunneling, which links proton transfer to two classes of protein motions: environmental reorganization (λ in Marcus-like equations) and protein fluctuations ("active dynamics"; gating) which modulate the distance of proton transfer.

V-B-2 Roles of Distal Asp in Heme Oxygenase from *Corynebacterium diphtheriae*, HmuO: A Water-Driven Oxygen Activation Mechanism

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[*J. Biol. Chem.* **280**, 2981–2989 (2005)]

Heme oxygenases found in mammals, plants and bacteria catalyze degradation of heme using the same mechanism. Roles of distal Asp (Asp136) residue in HmuO, a heme oxygenase of *Corynebacterium diphtheriae*, have been investigated by site-directed mutagenesis, enzyme kinetics, resonance Raman spectroscopy and X-ray crystallography. Replacements of the Asp136 by Ala and Phe resulted in reduced heme degradation activity due to the formation of ferryl heme, showing that the distal Asp is critical in HmuO heme oxygenase activity. D136N HmuO catalyzed heme degradation at a similar efficiency to wild type and D136E HmuO, implying that the carboxylate moiety is not required for the heme catabolism by HmuO. Resonance Raman results suggest that the inactive ferryl heme formation in the HmuO mutants is induced by disruption of the interaction between a reactive Fe–OOH species and an adjacent distal pocket water molecule. Crystal structural analysis of the HmuO mutants confirms partial disappearance of this nearby water in D136A HmuO. Our results provide the first experimental evidence for the catalytic importance of the nearby water molecule that can be universally critical in heme oxygenase catalysis, and propose that the distal Asp helps in positioning the key water molecule at a position suitable for efficient activation of the Fe–OOH species.

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

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[*J. Biol. Chem.* **280**, in press (2005)]

Heme oxygenase (HO) catalyzes the 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 in regulating HO activity. We have examined in detail the degradation of a synthetic verdoheme IXα complexed with rat HO-1. Our findings include: (1) HO degrades verdoheme through a dual pathway using either O_2 or H_2O_2; (2) the verdoheme reactivity with O_2 is the lowest among the three O_2 reactions in the HO catalysis, and the newly found H_2O_2 pathway is approximately 40-fold faster than the O_2-dependent verdoheme degradation; (3) both reactions are initiated by the binding of O_2 or H_2O_2 to allow the first direct observation of degradation intermediates of verdoheme; and (4) Asp140 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 Asp140. These mechanisms are similar to the 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. In addition, our results infer the possible involvement of the H_2O_2-dependent verdoheme degradation *in vivo*, and potential roles of the dual pathway reaction of HO against oxidative stress are proposed.

V-C Pro-Oxidants-Induced Iron Release from the Fe-S Cluster of Mitochondrial Aconitase and Its Prevention by Flataxin

Pro-oxidants, such as hydrogen peroxide and superoxide anion, are highly toxic for many living organisms. One of the adverse effects of pro-oxidants is modulation of mitochondrial respiration. Using EPR spectroscopy, we have identified that pro-oxidants deactivates mitochondrial aconitase, the key enzyme in citrate cycle, by releasing one of Fe from the 4Fe-4S cluster, and that mitochondria is surprisingly equipped a recovery mechanism to restore the active 4Fe-4S cluster. We have recently discovered that this restoration is achieved by an iron insertion from frataxin, an iron storage protein in mitochondria, which functions as an iron chaperon protein. We are in the process of elucidation of the inter protein iron transfer mechanism from frataxin to aconitase at molecular level.

V-C-1 Reversible Redox-Dependent Modulation of Mitochondrial Aconitase and Proteolytic Activity during *In Vivo* Cardiac Ischemia/Reperfusion

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[*Proc. Natl. Acad. Sci. U. S. A.* **102**, 5987–5991 (2005)]

Recent evidence indicates that mitochondrial aconitase can be reversibly inhibited or progress to irreversible inactivation and degradation in response to pro-oxidants. Cardiac ischemia/reperfusion is associated with an increase in mitochondrial free radical production. In the current study, the effects of reperfusion-induced production of pro-oxidants on mitochondrial aconitase and proteolytic activity were determined to assess whether alterations represented a regulated response to changes in redox status or oxidative damage. Evidence is provided that ATP-dependent proteolytic activity increased during early reperfusion followed by a time-dependent reduction in activity to control levels. These alterations in proteolytic activity paralleled an increase and subsequent decrease in the level of oxidatively modified protein. *In vitro* data supports a role for pro-oxidants in the activation of ATP-dependent proteolytic activity. Despite inhibition during early periods of reperfusion, aconitase was not degraded under the conditions of these experiments. Aconitase activity exhibited a decline in activity followed by reactivation during cardiac reperfusion. Loss and regain in activity involved reversible sulfhydryl modification. Aconitase was found to associate with the iron binding protein frataxin exclusively during reperfusion. *In vitro*, frataxin has been shown to protect aconitase from $[4Fe\text{-}4S]^{2+}$ cluster disassembly, irreversible inactivation, and potentially degradation. Thus, the response of mitochondrial aconitase and ATP-dependent proteolytic activity to reperfusion-induced pro-oxidant production appears to be a regulated event that would be expected to reduce irreparable damage to the mitochondria.

V-D Quantum Emissions from Solid in Femtosecond Intense Laser Field and Its Application to Dynamic Imaging

Quantum emissions, which are high-energy electron, ion and photon beams, generated by interaction of femtosecond intense laser field with matter has recently been attracting considerable attention because of interest in fundamental science and its potential applications in compact acceleration, proton therapy and materials sciences. We have studied a mechanism of quantum emissions from solid target and its application to dynamic imaging of materials.

V-D-1 Picosecond Time-Resolved X-Ray Diffraction from a Laser-Shocked Germanium Crystal over Hugoniot Elastic Limit

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[*Jpn. J. Appl. Phys.* **43**, 5477–5479 (2004)]

Picosecond time-resolved X-ray diffraction has been performed on a 300-ps laser-irradiated germanium crystal at 1.2 GW/cm^2. Lattice deformation due to shock compression and the propagation of shock waves are directly observed. The observed lattice compression is 4.3% at maximum, which is higher than that at the Hugoniot elastic limit (HEL). The data suggest that the germanium-crystal lattice behaves elastically under shock compression at 7.5 GPa (above HEL) for 27 ps.

V-D-2 Enhanced Generation of Fast Protons from a Polymer-Coated Metal Foil by a Femtosecond Intense Laser Field

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[*Appl. Phys. Lett.* **85**, 2736–2738 (2004)]

The results of generation of fast protons from 5-μm-thick copper foil targets by 60fs laser irradiation at 1.5 × 10^{17} W/cm^2 are presented. Both polyvinylmethylether (PVME)-coated and uncoated copper foil targets are examined. Fast protons are measured using a Thomson mass spectrometer and maximum proton energies are 570 and 280 keV for the PVME-coated and the uncoated target, respectively. The intensity of fast protons with energy of 160 keV from the PVME-coated target is approximately 80-fold higher than that from the uncoated target.

V-D-3 Electron Imaging of Charge Separated Field on a Copper Film Induced by Femtoseond Laser Irradiation

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[*Appl. Phys. Lett.* **86**, 141501–141503 (2005)]

An instantaneous charge-separated field, built up at the femtosecond-laser-irradiated surface of a copper film, was observed by time-resolved electron imaging using an energy-chirped electron probe-beam. The probe beams with effective energies of 170 keV were generated by intense femtosecond laser irradiation onto a molybdenum target at an intensity of 10^{17} W/cm^2. From the deflection of the probe electrons, the electric field was estimated to be 1.5 MV/m at a pump-laser intensity of 10^{15} W/cm^2.