X-F Biomolecular Science

Elucidation of a structure-function relationship of metalloproteins and structural chemistry of amyloid fibril are current subjects of this group. The primary technique used for the first project is the stationary and time-resolved resonance Raman spectroscopy excited by visible and UV lasers. Various model compounds of active site of enzymes are also examined with the same technique. IR-microscope dichroism analysis and AFM are the main techniques for the second project. The practical themes that we want to explore for the first project are (1) mechanism of oxygen activation by enzymes, (2) mechanism of active proton translocation and its coupling with electron transfer, (3) structural mechanism of signal sensing and transduction by heme-based sensory proteins, (4) higher order protein structures and their dynamics, and (5) reactions of biological NO. In category (1), we have examined a variety of terminal oxidases, cytochrome P450s (including AOS), and peroxidases, and also treated their reaction intermediates by using the mixed flow transient Raman apparatus and the Raman/absorption simultaneous measurement device. For (2) the third-generation UV resonance Raman (UVRR) spectrometer was constructed and we are applying it to a giant protein like cytochrome c oxidase with[M = 210,000, particularly to explore the oxidation state of Tyr244 in the Pₘ intermediate. Recently, we succeeded in pursuing protein folding of apomyoglobin by combining the UV time-resolved Raman and rapid mixing techniques. With IR spectroscopy we determined the spectrum of carboxylic side chains of bovine cytochrome oxidase which undergo protonation/deprotonation changes and hydrogen-bonding status changes in response with electron transfers between metal centers or ligand dissociation from heme a₃. In (3) we are interested in a mechanism of ligand recognition specific to CO, NO or O₂ and a communication pathway of the ligand binding information to the functional part of the protein. Several gas sensor heme proteins were extensively treated in this year. For (4) we developed a novel technique for UV resonance Raman measurements based on the combination of the first/second order dispersions of gratings and applied it successfully to 235-nm excited RR spectra of several proteins including mutant hemoglobin and myoglobin. Nowadays we can carry out time-resolved UVRR experiments with nanosecond resolution to discuss protein dynamics. With the system, we have succeeded in isolating the spectrum of tyrosinate in ferric Hb M Iwate, which was protonated in the ferrous state, and the deprotonated state of Tyr244 of bovine cytochrome c oxidase. The study is extended to a model of Tyr244, that is, imidazole-bound para-cresol coordinated to a metal ion, was synthesized and its UV resonance Raman was investigated. For (5) we purified soluble guanlylate cyclase from bovine lung and observed its RR spectra in the presence of allosteric effector, YC-1. The CO and NO adducts in the presence of YC-1 were examined. To further investigate it, we are developing an expression system of this protein.

For the amyloid study, we examined FTIR spectra of β₂-microglobulin and its fragment peptides of #11-21, K3, and K3-K7 which form a core part of amyloid fibril of β₂-microglobulin. The effect of seed upon the formation of the fibril was focused this year.

X-F-1 Resonance Raman Characterization of the P Intermediate in the Reaction of Bovine Cytochrome c Oxidase

OGURA, Takashi¹; KITAGAWA, Teizo
(¹IMS and Univ. Hyogo)


Reduced cytochrome c oxidase binds molecular oxygen, yielding an oxygenated intermediate first (Oxy) and then converts it to water via the reaction intermediates of P, F, and O in the order of appearance. We have determined the iron-oxygen stretching frequencies for all the intermediates by using time-resolved resonance Raman spectroscopy. The bound dioxygen in Oxy does not form a bridged structure with Cu₃ and the rate of the reaction from Oxy to P (P_R) is slower at higher pH in the pH range between 6.8 and 8.0. It was established that the P intermediate has an oxo-heme and definitely not the Fe₃-O-O-Cu₃ peroxo bridged structure. The Fe₃=O stretching frequency (v_Fe=O) of the Pₘ intermediate, 804/764 cm⁻¹ for ¹⁶O/¹⁸O, is distinctly higher than that of F intermediate, 785/750 cm⁻¹. The rate of reaction from P to F is quite different between H₂O and D₂O solutions, implicating the coupling of the electron transfer with vector proton transfer in this process. The P intermediate (607 nm form) generated in the reaction of oxidized enzyme with H₂O₂ gave the ν_Fe=O band at 803/769 cm⁻¹ and the simultaneously measured absorption spectrum exhibited the difference peak at 607 nm. Reaction of the mixed valence CO adduct with O₂ provided the P intermediate (Pₘ) giving rise to an absorption peak at 607 nm and the ν_Fe=O bands at 804/768 cm⁻¹. Thus, three kinds of P intermediates are considered to have the same oxo-heme a₃ structure. The ν₄ and ν₂ modes of heme a₃ of the P intermediate were identified at 1377 and 1591 cm⁻¹, respectively, and Raman excitation profiles were different between P and F. These observations may mean the formation of a π cation radical of porphyrin macrocycle in P.

X-F-2 Core Structure of Amyloid Fibril Proposed from IR-Microscope Linear Dichroism

HIRAMATSU, Hirotugu; GOTO, Yuji¹; NAIKI, Hironobu²; KITAGAWA, Teizo
(¹Osaka Univ.; ²Fukui Univ.)

[J. Am. Chem. Soc. 126, 3008–3009 (2004)]

A new approach for studying a peptide conformation of amyloid fibril has been developed. It is based on infrared linear dichroism analysis using an IR-microscope for aligned amyloid fibril. The polarization direct-
tions of amide I and II bands were perpendicular similarly for β₂-microglobulin and its #21-31 peptide. Furthermore, this approach has shown that the #21-31 peptide consists of two C=O bonds in the β-sheet that makes 0° with the fibril axis, three C=O bonds in the β-sheet inclined by 27° with respect to the fibril axis, four residues in the random coil by 47°, and two residues in possible β-bulge structure by 32°. Plausible structures of the amyloid core in the fibril are proposed by taking account of these results.

**X-F-3 Activation of Heme-Regulated Eukaryotic Initiation Factor 2α Kinase (HRI) Activation by Nitric Oxide Is Induced by the Formation of a Five-Coordinate NO-Heme Complex: Optical Absorption, Electron Spin Resonance and Resonance Raman Spectral Studies**

IGARASHI, Jotaro¹; SATO, Akira²; KITAGAWA, Teizo; YOSHIMURA, Tetsuhiko²; YAMAUCHI, Seigo¹; SAGAMI, Ikuko³; SHIMIZU, Toru¹
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[J. Biol. Chem. 279, 15752–15762 (2004)]

Heme-regulated eukaryotic initiation factor 2α kinase (HRI) regulates the synthesis of hemoglobin in reticulocytes in response to heme availability. HRI contains a tightly bound heme at the N-terminal domain. Earlier reports show that nitric oxide (NO) regulates HRI catalysis. However, the mechanism of this process remains unclear. In the present study, we utilize in vitro kinase assays, optical absorption, electron spin resonance (ESR), and resonance Raman spectra of purified full-length HRI for the first time to elucidate the regulation mechanism of NO. HRI was activated via heme upon NO binding, and the Fe(II)-HRI(NO) complex displayed 5-fold greater eukaryotic initiation factor 2α kinase activity than the Fe(III)-HRI complex. The Fe(III)-HRI complex exhibited a Soret peak at 418 nm and a rhombic ESR signal with g values of 2.49, 2.28, and 1.87, suggesting coordination with Cys as an axial amino group. Interestingly, optical absorption, ESR, and resonance Raman spectra of the Fe(II)-NO complex were characteristic of five-coordinate NO-heme. Spectral findings on the coordination structure of full-length HRI were distinct from those obtained for the isolated N-terminal hemebinding domain. Specifically, six-coordinate NO–Fe(II)–His was observed but not Cys-Fe(III)-HRI complex exhibited a Soret peak at 418 nm and a rhombic ESR signal with g values of 2.49, 2.28, and 1.87, suggesting coordination with Cys as an axial amino group. The steric repulsion of the coordination geometries around the metal centers are almost a trigonal-bipyramidal rather than a square-planar except for 1a with an intermediate between them. The UV-vis and ESR spectral data indicate that the increase of NH₂ groups of ligands causes the structural change from trigonal-bipiramidal to square-pyramidal geometry, which is regulated by a combination of steric repulsion and hydrogen bond. The steric repulsion of amino groups with the azide nitrogen gives rise to elongation of the Cu–N₃ bonds, which leads to the positive shift of the redox potentials of the complexes. The hydrogen bonds between the coordinated azide and amino nitrogens (2.84–3.05 Å) contribute clearly to the fixation of azide. The Cu(I) complexes with bapa and mapa ligands have been obtained as a precipitate, although that with tapa was not isolated. The reactions of the Cu(I) complexes with dioxygen in MeOH at ~75 °C have given the trans-μ-1,2 peroxo dinuclear Cu(II) complexes formulated as [{(tapa)Cu₂(O₂)]²⁺ (1c), [{(bapa)Cu₂(O₂)]²⁺ (2c), and [{(mapa)Cu₂(O₂)]²⁺ (3c), whose characterizations were confirmed by UV-vis, ESR, and resonance Raman spectroscopies. UV-vis spectra of 1c, 2c, and 3c exhibited intense bands assignable to π*(O₂²⁻)-to-d(Cu) charge transfer (CT) transitions at εmax/(εM⁻¹ cm⁻¹) = 449 (4620), 474 (6860), and 500 (9680), respectively. The series of the peroxo adducts generated was ESR silent. The resonance Raman spectra exhibited the enhanced features assignable to two stretching vibrations ν(O₁₆–O₁₈)/ν(O₁₆–O₁₈)/cm⁻¹ and ν(Cu₁₆O/Cu₁₈O)/cm⁻¹ at 835/807 (1c), 858/812 (2c), 847/800 (3c), and at 547/522 (2c), 544/518 (3c), respectively. The thermal stability of the peroxo-copper species has increased with increase in the number of the hydrogen-bonding interactions between the peroxide and amino groups.

**X-F-4 Steric and Hydrogen-Bonding Effects on the Stability of Copper Complexes with Small Molecules**

WADA, Akira¹; HONDA, Yasutaka¹; YAMAGUCHI, Syuhei³; NAGATOMO, Shigenori; KITAGAWA, Teizo; JITSUKAWA, Koichiro¹; MASUDA, Hideki²

(Nagoya Inst. Tech.; ²IMS and Nagoya Inst. Tech.)

[Inorg. Chem. 43, 5725–5735 (2004)]

A series of the copper(II) complexes with tripodal tetratadentate tri(pyridyl 2-methyl)amine-based ligands possessing the hydrogen-bonding 6-aminopyridine units (tapa, three amino groups; bapa, two amino groups; mapa, one amino group) have been synthesized, and their copper(II) complexes with a small molecule such as dioxygen and azide have been studied spectroscopically and structurally. The reaction of their Cu(II) complexes with NaN₃ have given the mononuclear copper complexes with diazide in an end-on mode, [Cu(tapa) (N₃)]ClO₄ (1a), [Cu(bapa)(N₃)]ClO₄ (2a), [Cu(mapa) (N₃)]ClO₄ (3a), and [Cu(tpa)(N₃)]ClO₄ (4a) (tpa, no amino group). The crystal structures have revealed that the coordination geometries around the metal centers are almost a trigonal-bipyramidal rather than a square-planar except for 1a with an intermediate between them. The UV-vis and ESR spectral data indicate that the increase of NH₂ groups of ligands causes the structural change from trigonal-bipiramidal to square-pyramidal geometry, which is regulated by a combination of steric repulsion and hydrogen bond. The steric repulsion of amino groups with the azide nitrogen gives rise to elongation of the Cu–N₃ bonds, which leads to the positive shift of the redox potentials of the complexes. The hydrogen bonds between the coordinated azide and amino nitrogens (2.84–3.05 Å) contribute clearly to the fixation of azide. The Cu(I) complexes with bapa and mapa ligands have been obtained as a precipitate, although that with tapa was not isolated. The reactions of the Cu(I) complexes with dioxygen in MeOH at ~75 °C have given the trans-μ-1,2 peroxo dinuclear Cu(II) complexes formulated as [{(tapa)Cu₂(O₂)]²⁺ (1c), [{(bapa)Cu₂(O₂)]²⁺ (2c), and [{(mapa)Cu₂(O₂)]²⁺ (3c), whose characterizations were confirmed by UV-vis, ESR, and resonance Raman spectroscopies. UV-vis spectra of 1c, 2c, and 3c exhibited intense bands assignable to π*(O₂²⁻)-to-d(Cu) charge transfer (CT) transitions at εmax/(εM⁻¹ cm⁻¹) = 449 (4620), 474 (6860), and 500 (9680), respectively. The series of the peroxo adducts generated was ESR silent. The resonance Raman spectra exhibited the enhanced features assignable to two stretching vibrations ν(O₁₆–O₁₈)/ν(O₁₆–O₁₈)/cm⁻¹ and ν(Cu₁₆O/Cu₁₈O)/cm⁻¹ at 835/807 (1c), 858/812 (2c), 847/800 (3c), and at 547/522 (2c), 544/518 (3c), respectively. The thermal stability of the peroxo-copper species has increased with increase in the number of the hydrogen-bonding interactions between the peroxide and amino groups.

**X-F-5 Identification of Crucial Histidines Involved in Carbon-Nitrogen Triple Bond Synthesis by Aldoxime Dehydratase**

KONISHI, Kazunobu¹; ISHIDA, Kyoko¹; OINUMA, Ken-Ichi³; OHTA, Takehiro; HASHIMOTO, Yoshihito²; HIGASHIBATA, Hiroyuki³; KITAGAWA, Teizo; KOBAYASHI, Michihiko¹
(¹Univ. Tsukuba)

[J. Biol. Chem. 279, 47619–47625 (2004)]
Aldoxime dehydratase (OxdA), which is a novel heme protein, catalyzes the dehydration of an aldoxime to a nitrile even in the presence of water in the reaction mixture. The combination of site-directed mutagenesis of OxdA (mutation of all conserved histidines in the aldoxime dehydratase superfamily), estimation of the heme contents and specific activities of the mutants, and CD and resonance Raman spectroscopic analyses led to the identification of the proximal and distal histidines in this unique enzyme. The heme contents and CD spectra in the far-UV region of all mutants except for the H299A one were almost identical to those of the wild-type OxdA, whereas the H299A mutant lost the ability of binding heme, demonstrating that His<sup>299</sup> is the proximal histidine. On the other hand, substitution of alanine for His<sup>320</sup> did not affect the overall structure of OxdA but caused loss of its ability of carbon-nitrogen triple bond synthesis and a lower shift of the Fe–C stretching band in the resonance Raman spectrum for the CO-bound form. Furthermore, the pH dependence of the wild-type OxdA closely followed the His protonation curves observed for other proteins. These findings suggest that His<sup>320</sup> is located in the distal heme pocket of OxdA and would donate a proton to the substrate in the aldoxime dehydration mechanism.

**X-F-6** Thermal Stability of Mononuclear Hydroperoxocopper(II) Species. Effects of Hydrogen Bonding and Hydrophobic Field

**YAMAGUCHI, Syuhei<sup>1</sup>; WADA, Akira<sup>2</sup>; NAGATOMO, Shigenori; KITAGAWA, Teizo; JITSUKAWA, Koichiro<sup>2</sup>; MASUDA, Hideki<sup>2</sup>  
<sup>1</sup>Nagoya Inst. Tech.; <sup>2</sup>IMS and Nagoya Inst. Tech.)

[Chem. Lett. 33, 1556–1557 (2004)]

The effects of hydrogen bonding and hydrophobic field on the thermal stabilities of Cu(II)–OOH complexes have been studied using tripodal tetradentate ligands with their functional groups on the basis of UV-vis, ESR, ESI-mass, and resonance Raman spectroscopies.

![Scheme 1](image)

We succeeded in obtaining a stable hydroperoxocopper(II) complex with a tripodal tetradentate ligand, bis(6-pivalamido-2-pyridylmethyl)(2-pyridylmethyl)amine (BPPA) (1h) for which the crystal structure and spectroscopic characterization of the hydroperoxocopper(II) complex were provided. Raman spectrum of 2h in acetonitrile measured at –40 °C (using 406.7 nm laser excitation) gave a resonance-enhanced Raman band at 850 cm<sup>–1</sup>, which shifted to 801 cm<sup>–1</sup> (Δν = 49 cm<sup>–1</sup>) when <sup>18</sup>O-labeled H<sub>2</sub>O<sub>2</sub> was used. That of 2h in methanol measured at –80 °C (using 406.7 nm laser excitation) gave Raman bands at 854 and 492 cm<sup>–1</sup>, assignable to ν(O–O) and ν(Cu–O), respectively. The formation of 2h was also confirmed from ESI mass spectrum measured in acetonitrile at –20 °C. The resonance Raman spectra of methanol solution of 3h measured at –80 °C (using 406.7 nm laser excitation) showed a resonance-enhanced Raman band at 847 and 512 cm<sup>–1</sup>, which are assigned to ν(O–O) and ν(Cu–O), the former of which shifted to 792 cm<sup>–1</sup> (Δν = 55 cm<sup>–1</sup>) when <sup>18</sup>O-labeled H<sub>2</sub>O<sub>2</sub> was employed. The formation of 3h was also confirmed from ESI mass spectrum measured in acetonitrile at –40 °C. Interestingly, the effects of hydrogen bonding and hydrophobic field on the thermal stabilities of Cu–OOH species, when their decomposition rates were followed using decrease in the absorption intensities of LMCT bands, was dramatically found out in the stability of these hydroperoxocopper(II) complexes.

**X-F-7** Energy Funneling of IR Photons Captured by Dendritic Antennae and Acceptor Mode Specificity: Anti-Stokes Resonance Raman Studies on Iron(III) Porphyrin Complexes with a Poly(Aryl Ether) Dendrimer Framework

MO, Yu-Jun<sup>1</sup>; JIANG, Donglin<sup>2</sup>; UYEMURA, Makoto<sup>2</sup>; AIDA, Takuzo<sup>2</sup>; KITAGAWA, Teizo<sup>2</sup>  
<sup>1</sup>IMS and Henan Univ.; <sup>2</sup>Univ. Tokyo)


A series of poly(aryl ether) dendrimer chloro-iron (III)porphyrin complexes (L<sub>n</sub>TPP)Fe(III)Cl (number of aryl layers [n] = 3 to 5) were synthesized and their Boltzman temperatures under IR irradiation were evaluated from ratios of Stokes to anti-Stokes intensities of resonance Raman bands. While the Boltzman temperature of neat solvent was unaltered by IR irradiation, (L<sub>n</sub>TPP)Fe(III)Cl (n = 3–5) all showed a temperature rise that was larger than that of the solvent and greater as the dendrimer framework was larger. Among vibrational modes of the metalloporphyrin core, the temperature rise of an axial Fe–Cl stretching mode at 355 cm<sup>–1</sup> was larger than that for a porphyrin in–plane mode at 390 cm<sup>–1</sup>. Although the most of IR energy is captured by the phenyl ν<sub>s</sub> mode at 1597 cm<sup>–1</sup> of the dendrimer framework, an anti-Stokes Raman band of the phenyl ν<sub>s</sub> mode was not detected, suggesting the extremely fast vibrational relaxation of the phenyl mode. From these observations, it is proposed that the energy of IR photons captured by the aryl dendrimer framework is transferred to the axial Fe–Cl bond of ironporphyrin core and then relaxed to the porphyrin macrocycle.

**X-F-8** Structural Model of the Amyloid Fibril Formed by β<sub>2</sub>-Microglobulin #21-31 Fragment Based on Vibrational Spectroscopy

HIRAMATSU, Hirosugu; GOTO, Yuji<sup>1</sup>; NAIKI, Hironobu<sup>2</sup>; KITAGAWA, Teizo<sup>2</sup>  
<sup>1</sup>Osaka Univ.; <sup>2</sup>Fukui Univ.)
A structural model of amyloid fibril formed by the #21-31 fragment of β2-microglobulin is proposed from microscope IR measurements on specifically 13C-labeled peptide fibrils and Raman spectra of the dispersed fibril solution. The 13C-shifted amide frequency indicated the secondary structure of the labeled residues. The IR spectra have demonstrated that the region between F22 and V27 forms the core part with the extended β-sheet structure. Raman spectra indicated the formation of a dimer with a disulfide bridge between C25 residues.

X-F-9 Excited State Property of Hardly Photodissociable Heme-CO Adduct Studied by Time-Dependent Density Functional Theory

OHTA, Takehiro; PAL, Biswajit; KITAGAWA, Teizo

While most of CO-bound hemes are easily photodissociated with a quantum yield of nearly unity, we occasionally encounter a CO-heme which appears hardly photodissociable under ordinary measurement conditions of resonance Raman spectra using CW laser excitation and a spinning cell. This study aims to understand such hemes theoretically, that is, the excited state properties of the five-coordinate heme-CO adduct (5cH) as well as the 6c heme-CO adduct (6cH) with a weak axial ligand. Using a hybrid density functional theory we scrutinized the properties of the ground and excited spin states of the computational models of a 5cH and a water ligated 6cH (6cH-H2O), and compared these properties with those of a photodissociable imidazole ligated 6cH (6cH-Im). Jahn-Teller softening for the Fe–C–O bending potential in the a1-e excited state was suggested. The excited state properties of 6cH-Im and 5cH were further studied with time-dependent DFT theory. The reaction products of the 6cH-Im and 5cH were assumed to be quintet and triplet states, respectively. According to the TD-DFT calculations, the Q excited state of 6cH-Im is a likely mechanism for apparent unphotodissociation.

X-F-10 Mechanism for Transduction of the Ligand-Binding Signal in Heme-Based Gas Sensory Proteins Revealed by Resonance Raman Spectroscopy

UCHIDA, Takeshi; KITAGAWA, Teizo

Gene analysis has revealed a variety of new heme-containing gas sensory proteins in organisms ranging from bacteria to mammals. These proteins are composed of sensor, communication, and functional domains. The sensor domain contains a heme that binds effector molecules such as NO, O2, or CO. Ligand binding by the sensor domain modulates the physiological role of the protein, such as DNA binding in the case of transcriptional factors or the catalytic reaction rate in the case of enzymes. This review summarizes resonance Raman (RR) studies, including static and time-resolved measurements, which have enabled elucidation of the mechanisms by which binding of specific target molecule by the sensor domain is transduced to alteration of the functional domain. These studies have shown that signals can be conveyed from the heme to the functional domain via three different pathways: i) a distal pathway, ii) a proximal pathway, and iii) a heme peripheral pathway.

X-F-11 UV Resonance Raman Study of Model Complexes of the CuII Site of Cytochrome c Oxidase

NAGANO, Yasutomo; LIU, Jin-Gang1; NARUTA, Yoshinori1; KITAGAWA, Teizo

A newly designed model complex for the CuII site of cytochrome c oxidase (CcO), that is, Cu coordinated by two free imidazoles and an imidazole covalently linked to p-cresol [CuII-BIAIPBr]Br, (BIAIP = 2-[[Bis(1-methyl-1H-imidazol-2-ylmethyl)amino)methyl]-1H-imidazol-1-yl]-4-methylphenol), and related molecules have been investigated with absorption and ultraviolet resonance Raman (UVRR) spectroscopy employing the excitation wavelengths between 220 and 290 nm. Attention was focused on the electron delocalization through the cross-linkage between the phenol and imidazole rings, and the influences by the coordination of CuII to imidazole. In addition to the ν3g and ν2g modes of p-cresol, a number of Raman bands involving vibrations of the imidazole moiety have been intensity-enhanced despite Raman excitation in resonance with the π-π* transition of phenol, indicating appreciable mixing of the π systems of imidazole and phenol rings. Furthermore, two kinds of imidazoles seem to be differential; one is the imidazole linked to p-cresol which yielded Raman bands at 1249, 1191, and 1141 cm⁻¹ for protonated CuII-BIAIP, and the other is one not linked to p-cresol, which yielded an intense band at 1488 cm⁻¹ band. Raman enhancement of the latter mode seems to be caused by preresonance to the lowest π-π* transition of imidazole via the A-term mechanism. The Raman excitation profile (REP) of ν2g mode for the deprotonated phenol of the CuII-complex revealed a weak local maximum corresponding to the L2 band around 240 nm. Raman enhancement by the L2 band was relatively weaker for the CuII-complex than for the ZnII-complex and metal-free ligand, suggesting the more extensive mixing of π systems of p-cresol-imidazole through the cross-linkage for the CuII-complex.


During the investigation of the absorption and ultraviolet resonance Raman (UVRR) properties of CuII-BIAIP, the CuII complex was studied. A structural model of amyloid fibril formed by the #21-31 fragment of β2-microglobulin was proposed from microscope IR measurements on specifically 13C-labeled peptide fibrils and Raman spectra of the dispersed fibril solution. The 13C-shifted amide frequency indicated the secondary structure of the labeled residues. The IR spectra demonstrated that the region between F22 and V27 forms the core part with the extended β-sheet structure. Raman spectra indicated the formation of a dimer with a disulfide bridge between C25 residues.

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X-F-12  Resonance Raman Investigation on the Specific Sensing Mechanism of a Target Molecule by Gas Sensory Proteins

OHTA, Takehiro; KITAGAWA, Teizo

[Inorg. Chem. 44, 758–769 (2005)]

Specific sensing of gas molecules such as CO, NO, and O₂ is a unique function of gas sensory hemoproteins, while hemoproteins carry out a wide variety of functions such as oxygen storage/transport, electron transfer, and catalysis as enzymes. It is important in the gas sensory proteins that the heme domain not only recognizes their target molecule, but also discriminates against other gases having similar molecular structures. Coordination of a target molecule to the heme is supposed to alter the protein conformation in the vicinity of heme, and the conformation change is propagated to the effector domain where substrate turnover, DNA binding, or interaction with a signal transduction protein will be performed in a way different from the case in binding of other gases. To understand the appearance of such a specificity, we focus our attention on the ligand-protein interactions in the distal side of heme here. Practically, the metal ligand vibrations as well as internal modes of ligand and heme are measured with resonance Raman spectroscopy for wild-type and some mutant proteins with full-length or limited sensory region. On the basis of such observations together with the knowledge currently available, we will discuss the mechanism of specific sensing of a diatomic molecule in gas sensory proteins.

X-F-13  Communication Pathway between Heme and Protein in Myoglobin

GAO, Ying1; EL-MASHTOLY, Samir F.; PAL, Biswajit; HAYASHI, Takashi2; HARADA, Katsuyoshi3; NAKAGAWA, Tomoyuki3; KITAGAWA, Teizo

(1SOKENDAI; 2IMS and Osaka Univ.; 3Kyushu Univ.)

[J. Am. Chem. Soc. submitted]

We investigated the communication pathway between heme and protein with sperm whale myoglobin as a model. It is known that Trp7 and Tyr151 exhibit UVRR spectral changes upon ligand binding to heme, we monitored their UVRR spectral changes for three kinds of proteins in which the plausible pathway was removed (i.e., via His93, propionate-6, or propionate-7). The UVRR results demonstrate that the absence of the H-bonds between propionate-7 and both Ser92 and His97 significantly perturbs the transduction of a structural change in heme to Trp7, but the cleavage of the Fe−His(93) covalent bond eliminates the communication to Tyr151. Thus the H-bonds between the propionate-7 and the F-helix regulate the conformational changes of the A helix, while the Fe−His bond is responsible for a change in the C-terminus but not for the A helix.

X-F-14  FT-IR Approaches on Amyloid Fibril Structure

HIRAMATSU, Hirotugu; KITAGAWA, Teizo

[Biochim. Biophys. Acta in press]

This review treats recent achievements of Fourier-transform infrared absorption spectroscopy on protein science, especially on amyloid fibril structure. It includes the brief explanation of theoretical background, description of related techniques, and recent applications to analysis of fibril structure. Concerns to theoretical background, successful analysis of Amide I in terms of transition dipole coupling between the C=O oscillators in peptide main chain has been described. The theory enables us to estimate a content of secondary structure in a protein. Related experimental techniques such as linear dichroism measurement, application of microscope, and isotope labeling, are introduced. The linear-dichroism measurement brings direct information on molecular orientation, microscope enables to treat a well-prepared particle, and isotope-label technique allows our structural discussion with one-residue resolution. Application of IR absorption spectroscopy and related techniques on amyloid fibril structure is reviewed. The model obtained is compared with protein native structure.


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(µ-Hydroxo or oxo)(µ-1,2-peroxo)diiron(III) complexes having a tetradentate tripodal ligand (L) containing a carboxylate sidearm [Fe₂(µ-OH or µ-O)(µ-O₂)(L)]⁺ were synthesized as models for peroxo-intermediates of non-heme diiron proteins and characterized by various physicochemical measurements including X-ray analysis, which provide fundamental structural and spectroscopic insights into the peroxodiiron(III) complexes.

X-F-16  Axial Ligand Substituted Nonheme FeV≡O complexes: Observation of Near-UV LMCT Bands and Fe≡O Raman Vibrations

SASTRI, Chivukula V.1; PARK, Mi Joo1; OHTA, Takehiro; JACKSON, Timothy A.2; STUBNA,
Axial ligand substitution of a mononuclear nonheme oxoiron(IV) complex, [Fe^{IV}(O)(TMC)(NCCH\textsubscript{3})\textsuperscript{2+}] (TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetracane), leads to the formation of new Fe^{IV}=O species with relatively intense electronic absorption features in the near-UV region. The presence of these near-UV features allowed us to make the first observation of Fe=O vibrations of S = 1 mononuclear nonheme oxoiron(IV) complexes by resonance Raman spectroscopy. We have also demonstrated that the reactivity of nonheme oxoiron(IV) intermediates is markedly influenced by the axial ligands.

A new tetradentate tripodal ligand (L3) containing sterically bulky imidazolyl groups was synthesized, where L3 is tris(1-methyl-2-phenyl-4-imidazolylmethyl) amine. Reaction of a bis(µ-hydroxy)dicopper(II) complex, [Cu\textsubscript{2}(L3)(OH)\textsubscript{2}]\textsuperscript{2+} (1), with H\textsubscript{2}O\textsubscript{2} in acetonitrile at ~−40 °C generated a (µ-1,1-hydroperoxy)dicopper(II) complex [Cu\textsubscript{2}(L3)(OOH)(OH)]\textsuperscript{2+} (2), which was characterized by various physicochemical measurements including X-ray crystallography. The crystal structure of 2 revealed that the complex cation has a Cu\textsubscript{2}(µ-1,1-OOH)(µ-ΩH) core and each copper has a square pyramidal structure having an N\textsubscript{2}O\textsubscript{2} donor set with a weak coordination, which produces a hydrophobic cavity around the Cu\textsubscript{2}(µ-1,1-OOH)(µ-ΩH) core. The hydrophobic cavity is preserved by hydrogen bondings between the hydroperoxide and the imidazole nitrogen of an uncoordinated pendant arm in one side and the hydroxide and the imidazole nitrogen of an uncoordinated pendant arm in the other side. The hydrophobic cavity significantly suppresses the H/D and \textsuperscript{18}O/\textsuperscript{16}O exchange reactions in 2 compared to that in 1 and stabilizes the Cu\textsubscript{2}(µ-1,1-OOH)(µ-ΩH) core against decomposition. Decomposition of 2 in acetonitrile at 0 °C proceeded mainly via disproportionation of the hydroperoxy ligand and reduction of 2 to [Cu(L3)]\textsuperscript{+} by hydroxyperoxo ligand. In contrast, decomposition of a solid sample of 2 at 60 °C gave a complex having a hydroxylated ligand [Cu\textsubscript{2}(L3)(L3-OH)(OH)]\textsuperscript{2+} (2-(L3-OH)) as a main product, where L3-OH is an oxidized ligand in which one of the imidazole nitrogen of an uncoordinated pendant arm, with the arene hydroxylation reported for some (µ-1,1-hydroperoxy)dicopper(II) complexes with a xylyl linker.

### X-F-17 Reversible O–O Bond Cleavage and Formation of a Peroxo Moiet of a Peroxocarbonate Ligand Mediated by an Iron(III) Complex

FURUTACHI, Hideki\textsuperscript{1}; HASHIMOTO, Koji\textsuperscript{1}; NAGATOMO, Shigenori; ENDO, Taichi\textsuperscript{1}; FUJINAMI, Shuhei\textsuperscript{1}; WATANABE, Yoshihito\textsuperscript{2}; KITAGAWA, Teizo; SUZUKI, Masatatsu\textsuperscript{1} (\textsuperscript{1}Kanazawa Univ.; \textsuperscript{2}IMS and Nagoya Univ.)

[A. Chem. Soc. 127, 4550–4551 (2005)]

A mononuclear iron(III) complex containing a peroxocarbonate ligand, [Fe\textsubscript{2}(q)\textsubscript{2}(O\textsubscript{2}C(O)\textsubscript{2})\textsuperscript{2+} (q = quinaldinate), underwent the reversible O–O bond cleavage and reformation of the peroxo group via the formation of Fe\textsuperscript{IV}=O or Fe\textsuperscript{V}=O species, which was confirmed by the resonance Raman and ESI-TOF/MS measurements.

### X-F-18 Synthesis and Reactivity of a (µ-1,1-Hydroperoxy)(µ-Hydroxy)Dicopper(II) Complex: Ligand Hydroxylation by a Bridging Hydroperoxo Ligand

ITOH, Kyosuke\textsuperscript{1}; HAYASHI, Hideo\textsuperscript{1}; FURUTACHI, Hideki\textsuperscript{1}; MATSUMOTO, Takahiro\textsuperscript{1}; NAGATOMO, Shigenori; TOSHA, Takehiko; TERADA, Shoji\textsuperscript{1}; FUJINAMI, Shuhei\textsuperscript{1}; SUZUKI, Masatatsu\textsuperscript{1}; KITAGAWA, Teizo (\textsuperscript{1}Kanazawa Univ.)

[A. Chem. Soc. 127, 5212–5223 (2005)]
were the axial ligands of the Fe(III) and Fe(II) hemes in Ch-CooA and that the N-terminal α-amino group was replaced by CO upon CO binding. Two neutral ligands, His-82 and the N-terminal α-amino group, are coordinated to the Fe(III) heme in Ch-CooA, whereas two negatively charged ligands, a thiolate from Cys-75 and the nitrogen atom of the N-terminal Pro, are the axial ligands of the Fe(III) heme in Rr-CooA. The difference in the coordination structure of the Fe(III) heme resulted in a large positive shift of redox potentials of Ch-CooA compared with Rr-CooA. Comparing the properties of Ch-CooA and Rr-CooA demonstrates that the essential elements for CooA function will be: (i) the heme is six-coordinate in the Fe(III), Fe(II), and Fe(II)–CO forms; (ii) the N-terminal is coordinated to the heme as an axial ligand; and (iii) CO replaces the N-terminal bond to the heme upon CO binding.

**X-F-20 Structural Diversities of Active Site in Clinical Azole-Bound Forms between Sterol 14α-Demethylases (CYP51s) from Human and Mycobacterium tuberculosis**

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To gain insights into the molecular basis of the design for the selectiveazole anti-fungals, we compared the binding properties ofazole-based inhibitors for cytochrome P450 sterol 14α-demethylase (CYP51) from human (HuCYP51) and Mycobacterium tuberculosis (MtCYP51). Spectroscopic titration of azoles to the CYP51s revealed that HuCYP51 has higher affinity for ketoconazole (KET), an azole derivative that has long hydrophilic groups, than MtCYP1, but the affinity for fluconazole (FLU), which is a member of the anti-fungal armamentarium, was lower in HuCYP51. The affinity for 4-phenylimidazole (4-PhIm) to MtCYP1 was shifted to high frequency as the prominent high frequency shift of the bending mode of the heme propionate group for the FLU-bound MtCYP1 and FLU-bound HuCYP1 gave shifted to low frequency. The EPR spectra for 4-PhIm-bound MtCYP1 was shifted to high frequency as the prominent high frequency shift of the bending mode of the heme propionate group for the FLU-bound MtCYP1 and FLU-bound HuCYP1 gave shifted to low frequency. The EPR spectra for 4-PhIm-bound MtCYP1 and FLU-bound HuCYP1 gave multiple g values, showing heterogeneous binding of the azoles, whereas the single g$_x$ and g$_z$ values were observed for other azole-bound forms. Together with the alignment of the amino acid sequence, these spectroscopic differences suggest that the region between the B' and C helices, particularly the hydrophobicity of the C helix, in CYP51s plays primary roles in determining strength of interactions with azoles; this differentiates the binding specificity of azoles to CYP51s.

**X-F-21 Stopped-Flow Spectrophotometric and Resonance Raman Analyses of Aldoxime Dehydratase Involved in Carbon-Nitrogen Triple Bond Synthesis**

OINUMA, Ken-Ichi; KUMITA, Hideyuki; OHTA, Takehiro; KONISHI, Kazunobu; HASHIMOTO, Yoshiteru; HIGASHIBATA, Hiroki; KITAGAWA, Teizo; SHIRO, Yoshitsugu; KOBAYASHI, Michihiko (1 Univ. Tsukuba; 2 RIKEN Harima Inst./SPring-8)

On stopped-flow analysis of aliphatic aldoxime dehydratase (OxdA), a novel hemoprotein, a spectrum derived from a reaction intermediate was detected on mixing ferrous OxdA with butyraldoxime; it gradually changed into that of ferrous OxdA with an isosbestic point at 421 nm. The spectral change on the addition of butyraldoxime to the ferrous H320A mutant showed the formation of a substrate-coordinated mutant, the absorption spectrum of which closely resembled that of the above intermediate. These observations and the resonance Raman investigation revealed that the substrate actually binds to the heme in OxdA, forming a hexa-coordinate low-spin heme.

**X-F-22 Synthesis, Characterization, and Thermal Stability of New Mononuclear Hydrogenperoxocopper(II) Complexes with N$_2$O-Type Tripodal Ligands Bearing Hydrogen-Bonding Interaction Sites**

YAMAGUCHI, Syuhei; KUMAGAI, Akinori; NAGATOMO, Shigenori; KITAGAWA, Teizo; FUNAHASHI, Yasuhiro; OZAWA, Tomohiro; JITSUKAWA, Koichiro; MASUDA, Hideki (1 Nagoya Inst. Tech.; 2 IMS and Nagoya Inst. Tech.)

In order to understand the effect of an oxygen-containing ligand on the physico-chemical properties and reactivities of hydrogenperoxocopper complexes, new copper(II) complexes with the N$_2$O-type tripodal ligand bearing pivalamido groups, N,N-bis(6-pivalamido-2-pyridylmethyl)glycine (Hbppga), and N,N-bis(6-pivalamido-2-pyridylmethyl)-β-alanine (Hbpaaa), have been designed and synthesized. Copper(II) complexes without any external ligand and those with a monodentate ligand, such as azido and chloro, have been prepared and characterized with the aid of electronic absorption and ESR spectroscopic, cyclic voltammetric, and X-ray structure analytical methods. The redox potential values of the Cu(II) complexes, when they were compared with the Cu(II) complex of bis(6-pivalamido-2-pyridylmethyl)amine (bpa), reported previously, shifted toward the negative side upon the introduction of a carboxylate group in the place of one pyridine of bpa. Reactions of [Cu(bpga)]ClO$_4$ (1a) and [Cu(bpaa)]PF$_6$ (2a) with hydrogen peroxide in the presence of triethylamine in both MeCN and MeOH solutions gave mononuclear copper(II) com-
plexes with hydrogenperoxide(1-), Cu–bpga–OOH (1d) and Cu–bpa–OOH (2d) systems, respectively. The intense absorption bands, assignable to LMCT (HOO→Cu(II)) and d–d bands, and ESR and resonance Raman spectra have revealed that they form trigonal bipyramidal copper complexes with OOH− in an end-on fashion. The thermal stabilities of 1d and 2d have also been studied by following the reduction rate of the LMCT bands at 283 K. Those of copper(II) complexes with hydrogenperoxide(1-) have been reduced in the order 1d > 2d >> [Cu(bppa)(OOH)]+ (3d), all of which are rather stable compared with that of Cu(II)–tpa–OOH (tpa = tris(2-pyridylmethyl)amine). These findings indicate that the hydrogenperoxo-copper(II) complexes are activated by introducing carboxylate coordination, although they are stabilized by hydrogen-bonding interactions.

**X-F-23 Spectroscopic Characterization of the Isolated Heme-Bound PAS-B Domain of Neuronal PAS Domain Protein 2 (NPAS2) Associated with Circadian Rhythms**

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[FEBs J. 272, 4153–4162 (2005)]

Neuronal PAS domain protein 2 (NPAS2) is an important transcription factor associated with circadian rhythms. This protein forms a heterodimer with BM AL1, which binds to the E-box sequence to mediate circadian rhythm-regulated transcription. NPAS2 has two PAS domains with heme-bonding sites in the N-terminal portion. In this study, we overexpressed wild-type and His mutants of the PAS-B domain (residues 241–416) of mouse NPAS2 and then purified and characterized the isolated hemebound proteins. Optical absorption spectra of the wild-type protein showed that the Fe(III), Fe(II) and Fe(II)–CO complexes are 6-coordinated low-spin complexes. On the other hand, resonance Raman spectra indicated that both the Fe(III) and Fe(II) complexes contain mixtures of 5-coordinated high-spin and 6-coordinated low-spin complexes. Based on inverse correlation between νFe-CO and νC=O of the resonance Raman spectra, it appeared that the axial ligand trans to CO of the heme-bound PAS-B is His. Six His mutants (His266Ala, His289Ala, His300Ala, His302Ala, His329Ala, and His335Ala) were generated, and their optical absorption spectra were compared. The spectrum of the His335Ala mutant indicated that its Fe(III) complex is the 5-coordinated high-spin complex, whereas, like the wild-type, the complexes for the five other His mutants were 6-coordinated low-spin complexes. Thus, our results suggest that one of the axial ligands of Fe(III) in PAS-B is His335. Also, binding kinetics suggest that heme binding to the PAS-B domain of NPAS2 is relatively weak compared with that of sperm whale myoglobin.

**X-F-24 Covalent Cofactor Attachment to Proteins: Cytochrome c Biogenesis**

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[Biochem. Soc. Trans. 33, 792–795 (2005)]

Haem (Fe-protoporphyrin IX) is a cofactor found in a wide variety of proteins. It confers diverse functions, including electron transfer, the binding and sensing of gases, and many types of catalysis. The majority of cofactors are non-covalently attached to proteins. There are, however, some proteins in which the cofactor binds covalently and one of the major protein classes characterized by covalent cofactor attachment is the c-type cytochromes. The characteristic haem-binding mode of c-type cytochromes requires the formation of two covalent bonds between two cysteine residues in the protein and the two vinyl groups of haem. Haem attachment is a complex post-translational process that, in bacteria such as *Escherichia coli*, occurs in the periplasmic space and involves the participation of many proteins. Unexpectedly, it has been found that the haem chaperone CcmE (cytochrome c maturation), which is an essential intermediate in the process, also binds haem covalently before transferring the haem to apocytochromes. A single covalent bond is involved and occurs between a haem vinyl group and a histidine residue of CcmE. Several in vitro and in vivo studies have provided insight into the function of this protein and into the overall process of cytochrome c biogenesis.

**X-F-25 Structure and Dioxygen-Reactivity of Copper(I) Complexes Supported by Bis(6-methylpyridin-2-yl-methyl)amine Tridentate Ligands**

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[Dalton Trans. in press]

Structure and dioxygen-reactivity of copper(I) complexes 2R supported by N,N-bis(6-methylpyridin-2-yl-methyl)amine tridentate ligands L2R[R (N-alkyl substituent) = –CH3Ph (Bn), –CH2CH2Ph (Phe) and –CH2CHPh2 (PhePh)] have been examined in comparison with those of copper(I) complex 1Phe of N,N-bis[2-(pyridin-2-yl)ethyl]amine tridentate ligand L1Phe and copper(I) complex 3Phe of N,N-bis(pyridin-2-yl-methyl)amine tridentate ligand L3Phe. Copper(I) complexes 2Phe and 2PhePh exhibit a distorted trigonal pyramidal structure involving a d-π interaction with an η1-binding mode between the metal ion and one of the ortho-carbon atoms of the phenyl group of the ligand side arm [–CH2CH2Ph (Phe) and –CH2CHPh2 (PhePh)]. Strength of the d-π interaction in 2Phe and 2PhePh is weaker than that of the d-π interaction with an η1-binding mode in 1Phe but stronger than that of the η1 d-π interaction in...
The cytochrome c maturation protein CcmE is an essential membrane-anchored heme chaperone involved in the post-translational covalent attachment of heme to c-type cytochromes in Gram-negative bacteria such as Escherichia coli. Previous in vitro studies have shown that CcmE can bind heme both covalently (via a histidine residue) and non-covalently. In this work we present results on the latter form of heme binding to a soluble form of CcmE. Examination of a number of site-directed mutants of E. coli CcmE by resonance Raman spectroscopy has identified ligands of the heme iron and provided insight into the initial steps of heme binding by CcmE before it binds the heme covalently. The heme-binding histidine (H130) appears to ligate the heme iron in the ferric oxidation state but two other residues ligate the iron in the ferrous form, thereby freeing H130 to undergo covalent attachment to a heme vinyl group. It appears that the heme ligation in the non-covalent form is different from that in the holo-form, suggesting that a change in ligation could act as a trigger for the formation of the covalent bond.