dation by cleaving between threonine and glycine at an LPXTG (X=D, E, A, N, Q, K) recognition motif and subsequently joining the carboxyl group of threonine to an amino group of pentaglycine on the cell wall peptidoglycan. In vitro, sortase slowly hydrolyzes an LPXTG peptide in the absence of a suitable nucleophile, but catalyzes exclusively a transpeptidation in the presence of a triglycine. Nitric oxide synthase (eNOS), possessing a C-terminal LPETG motif, was used as a target protein. The protein was allowed to react with a fluorescently labeled peptide, GGGGC-Alexa Fluora 546, for up to 8 hours. The reaction products, with or without sortase, were analyzed on SDS-PAGE. Spectrophotometric analysis demonstrated that stoichiometric amount of fluorophore was incorporated into the protein. Conformational change of eNOS upon binding with $\text{Ca}^{2+}/\text{CaM}$ was probed by fluorescence energy transfer from flavin in eNOS to the attached AlexaFluora 546. Fluorescence spectrum of eNOS labeled with AlexaFluora 546 was measured with the excitation at flavin absorption. Addition of $\text{Ca}^{2+}/\text{CaM}$ enhanced the fluorescence intensity due to AlexaFluora-546 with concomitant loss of fluorescence intensity due to flavin. Enhancement of energy transfer rate indicates that $\text{Ca}^{2+}/\text{CaM}$ binding induces conformational change of eNOS so as to change the distance between two fluorophores.

1P107 チトクロム *c* 酸化酵素の CO 光解離後の構造緩和：ヘム側鎖の 1G1340 ラマンバンドの帰属

Structural Relaxation of Cytochrome *c* Oxidase after CO Photolysis: Assignment of a Heme Peripheral Raman Mode

Izumi Ishigami¹, Takeshi Nishigaki¹, Satoru Nakashima², Kyoko Shinzawa-Itoh¹, Shinya Yoshikawa^{1,2}, Takashi Ogura^{1,2}, (¹University of Hyogo, Grad.Sch. Life Sci.; ²Picobiology Inst., Grad.Sch. Life Sci., University of Hyogo)

Cytochrome *c* oxidase (CcO) reduces dioxygen to water, and translocates protons from the negative to the positive spaces of the inner mitochondrial membrane. CcO has two hemes, namely, heme *a*₃ and heme *a*. Helix X near two hemes exhibits conformational change upon redox-change and ligand binding of heme *a*₃, and contains His376 and His378 as axial ligands to hemes *a*₃ and *a*.

In this project, we followed the relaxation process from the CO-bound to the equilibrium reduced state of CcO after CO photolysis with time-resolved resonance Raman spectroscopy with delay time between 10 ns and 5 ms. We have reported that the Fe-His bond strength of heme *a*₃ exponentially weakened with a rate constant of $1.3 \times 10^6 \text{ s}^{-1}$, the dynamics of which was assignable to the movement of helix X and that a Raman band at 435 cm^{-1} for CO-bound form exhibited an upshift at delay time later than 5 ms. The mode was deduced to be a bending mode of either formyl or vinyl substituent of heme *a*₃. However, the assignment of the band at 435 cm^{-1} was not established, although it was reported that the bend was deuteration sensitive.

In order to distinguish the two possibilities, we examined the time dependence of the $\nu(\text{CH}=\text{O})$ mode (formyl) at 1667 cm^{-1} and the $\nu(\text{C}=\text{C})$ mode (vinyl) at 1626 cm^{-1} with the same technique. Based on the results, the band at 435 cm^{-1} is assignable to the vinyl substituent.

1P108 共鳴ラマン分光法によるチトクロム *c* 酸化酵素のヘム間相互作用の検出 1G1355

Resonance Raman Evidence for the Heme-heme interaction in Cytochrome *c* Oxidase

Miyuki Sakaguchi, Kyoko Shinzawa-Itoh, Shinya Yoshikawa, Takashi Ogura, (University of Hyogo)

Cytochrome *c* oxidase is the terminal enzyme of the respiratory chain in mitochondrial inner membrane. X-ray crystallography finally demonstrated the existence of the two different kinds of heme. CcO possesses four redox active metal centers, namely, Cu_A , heme *a*, heme *a*₃ and Cu_B . Heme *a*₃ and Cu_B serves as a binuclear center to catalyze the reduction reaction of molecular oxygen. Heme *a* is suggested to drive proton-pumping and coupling mechanism of oxygen reduction and the proton-pumping is a current issue. Helix X in CcO contains H376 and H378, which are the axial ligands to heme *a*₃ and heme *a*, respectively, and the conformation of helix X close to the two axial ligands is controlled by the oxidation and ligand-binding state of heme *a*₃.

In the present study, we have examined the structural change of heme *a*₃ upon the redox change of heme *a*. In the experiments, we fixed heme *a*₃ in the Fe(II)-CO or Fe(III)-CN⁻ state and examined the effects of oxidation state change of heme *a* by probing the vibrational modes of heme *a*₃ with resonance Raman spectroscopy. In both cases, the formyl $\text{CH}=\text{O}$ stretching and the Fe-ligand stretching modes of heme *a*₃ exhibited significant frequency shifts upon redox change of heme *a*. The results have thus demonstrated that there exists a heme *a*-heme *a*₃ interaction. Considering such interaction might help us to clarify unsolved problems in the mechanisms of oxygen reduction, proton-pumping and their coupling.

1P109 フェムト秒レーザーを用いた高感度時間分解赤外分光装置の開発 発：酵素反応の生理条件下での測定を目指して Femtosecond-Laser-Based Highly-Sensitive Time-Resolved IR Spectrometer for Studying Enzyme Reactions Under Physiological Conditions

Minoru Kubo¹, Satoru Yamaguchi², Masao Mochizuki¹, Shinya Yoshikawa^{1,2}, Takashi Ogura^{1,2}, Satoru Nakashima¹, (¹Picobiology Inst., Grad. Sch. Life Sci., Univ. Hyogo; ²Dept. Life Sci., Grad. Sch. Life Sci., Univ. Hyogo)

A time-resolved IR spectrometer with a femtosecond mid-IR laser was developed to probe reaction dynamics of enzymes under physiological conditions. IR pulse was produced by a difference frequency generator with an optical parametric amplifier, which was pumped by the output of a regeneratively amplified Ti:sapphire laser system. The combination of tunability (3-16 μm), ultrawide bandwidth (500 cm^{-1}), high energy (10 μJ), good stability (<5 %), and high repetition rate (1 kHz) makes this laser system suitable for IR spectroscopy of enzymes in aqueous solution. In this spectrometer, the IR light was split into a probe arm and a reference arm, the latter of which was used for monitoring every pulse energy. After transmission through the sample, both the beams were dispersed in a spectrograph and imaged onto a double detector array (2x64) of MCT elements. The detector signals were sampled on a single shot basis, to compensate for pulse-to-pulse energy fluctuations and to perform statistical analysis and filtering of the data. The sample was placed in a spinning cell or a continuous flow cell, with a light path length of 50 μm . This system was tested on carbonmonoxy cytochrome *c* oxidase in H_2O buffer with pumping at 532 nm for CO photolysis. Nanosecond time-resolved signals of the Fe_{a3} - and Cu_B -bound CO stretching modes were successfully observed with a data acquisition time of only 10 sec. The measurements to detect the protein structural relaxation that occurs on a μs time scale is currently underway.

1P110 Resonance Raman study on the oxygenated and ferryl-oxo species of indoleamine 2,3-dioxygenase

Sachiko Yanagisawa¹, Hiroshi Sugimoto², Yoshitsugu Shiro², Takashi Ogura¹, (¹Univ. of Hyogo, Picobiology Institute; ²RIKEN SPring-8 Center, Harima Institute)

Indoleamine 2,3-dioxygenase (IDO) is a heme-containing dioxygenase found in several different mammalian tissues, but liver. This enzyme catalyzes a dioxygenation reaction of L-tryptophan (Trp) in the production of N-formylkynurenine. This is the main catabolic pathway of Trp. The mechanism of the dioxygenation reaction is unknown, because the structures of the intermediates have not been characterized. Very recently, resonance Raman (RR) spectra of the oxygenated and ferryl-oxo ($\text{Fe}^{IV}=\text{O}$) reaction intermediates of IDO were reported^{1,2}. Since the $\text{Fe}^{IV}=\text{O}$ reaction intermediate was observed in a condition of low enzymatic activity, it remains controversial whether $\text{Fe}^{IV}=\text{O}$ species is active intermediate or not. Here we report absorption and RR spectra, which reveal that the electronic and geometric structures of the two respective species at pH 6.5 and pH 8.0 are the same, although the enzymatic activity at pH 6.5 is 6 times higher than at pH 8.0. The results thus further support our current understanding that the $\text{Fe}^{IV}=\text{O}$ heme species is the active species in the IDO reaction cycle, although its presence was unexpected. The Fe-O₂ and the O-O stretching frequencies of the IDO-Trp-O₂ ternary complex at Trp concentrations of 50 μM and 8 mM are essentially identical. The results suggest that "substrate inhibition" of enzymatic activity occurs by binding of a second substrate molecule to an unknown binding site and not to the heme pocket.

¹Yanagisawa *et al.*, *Chem. Lett.*, **2010**, 39, 36. ²Lewis-Ballester *et al.*, *PNAS*, **2009**, 106, 17371.

1P111 中温性及び好熱性シトクローム *c* の熱安定性に及ぼすヘムの効果：実験結果と理論解析結果の比較

Effects of heme on the thermal stability of cytochromes *c*: Comparison between experimental and theoretical results

Ryota Kodama¹, Takashi Yoshidome¹, Masaru Yamanaka², Yoshihiro Sambongi², Masahiro Kinoshita¹, (¹Institute of Advanced Energy, Kyoto University, Uji, Kyoto 611-0011, Japan; ²Graduate School of Biosphere Science, Hiroshima University, Hiroshima 739-8528, Japan)

We have proposed a measure of the thermal stability of a protein [1]: the water-entropy gain at 298 K upon folding normalized by the number of residues, which is calculated using a hybrid of the angle-dependent integral equation theory and the morphometric approach. We extend the study to analyses on the effects of heme on the thermal stability of four cytochromes *c* (PA_{c551}, PH_{c552}, HT_{c552}, and AA_{c555}) whose denaturation temperatures are considerably different from one another despite that they share significantly high sequence homology and similar three-dimensional folds [2,3]. The major conclusions are as follows. The thermal stability is largely enhanced by the heme binding