2P091 **チトクローム酸化酵素の反応初期過程における共役機構の解明****Elucidation of the coupling mechanism in the initial stage of the reaction of cytochrome c oxidase**

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CO photolysis of Cytochrome c oxidase has been studied by using time-resolved IR and resonance Raman spectroscopy. It was observed that multi parts of the protein cooperate each other to attain its proton pumping function. Especially, for time-resolved IR spectroscopy, we developed a new IR spectrometer, which makes it possible to pursue the protein reaction in water solution under physiological conditions. From the observed results, the ligand attached to CuB site just after the photodissociation, and escaped toward bulk solution within 2 μ sec. Accompanied with this ligand behavior, the iron-His bond became weak, and α -helix structure along proton pump pathway, changed its structure to close the gate of the water channel, which protects the back leak of protons.

2P092 **過渡吸収分光法による一酸化窒素還元酵素の単寿命 NO 結合体の解析****Characterization of transient NO-bound form of Nitric Oxide Reductase by Transient Absorption Spectroscopy**

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Nitric oxide reductase (NOR) is a membrane protein, which reduces NO to N₂O at the catalytic center consisting of heme *b* and non-heme Fe_B. Although the clarification of the transient NO-bound form is critical to understand the molecular mechanism of NO reduction, capturing this early intermediate is difficult because of the fast NO reduction (μ s to ms). To observe the NO-bound form in real-time manner, we developed the time-resolved visible (TR-vis) and infrared (TR-IR) spectroscopic measurement systems, in which caged-NO is used to trigger the reaction. TR-vis spectroscopy showed that the NO-bound intermediate formed within 4 μ s. The further characterization of NO-bound form by TR-IR, which monitors the N-O stretching vibrational mode, is now underway.

2P093 **チトクロム c 酸化酵素の酸素還元反応の時間分解赤外吸収測定を目的とした酸素肺フローシステムの開発****Development of sample flow system with an oxygen lung for time-resolved infrared measurements of cytochrome c oxidase**

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A new flow system aimed at measuring time-resolved (TR) IR spectra of the oxygen reduction reaction by cytochrome c oxidase (CcO) in aqueous solution was developed. Oxygen is supplied by the inserted artificial oxygen lung. The reaction is triggered by CO photolysis of CO-bound CcO. To measure the IR spectra for aqueous solution, the path length of the flow cell must be made extremely thin (50 μ m) to reduce high absorbance background of water and, in this case, smooth flowing of the solution should be warranted. To evaluate the system, TR visible absorption spectra were observed. Since the reported intermediates and their dynamics were well reproduced, proper functioning of the system was confirmed. Currently, we are trying to measure TR IR spectra with this system.

2P094 **タンパク質を基盤とした酸素濃度 FRET センサー**
Protein-based FRET sensor for oxygen concentration

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The sea lamprey hemoglobin (slHb) is primarily monomeric in oxy form, while slHb self-associates to dimers or tetramers upon deoxygenation. The oxygen dependent monomer-dimer transition of slHb is useful to apply to the FRET approach. The single cysteine residues in slHb were labeled by Alexa Fluor 488 or 594. Purified slHb with Alexa Fluor dyes was mixed and measured by the fluorescence spectroscopy. There was no FRET in our FRET system when the oxygen concentration is high. On the other hand, the labeled slHb exhibited FRET signal under low oxygen concentration. Since slHb self-associates upon deoxygenation, slHb would bring the Alexa Fluor dye in close enough proximity to allow for FRET. Our FRET system could be useful to detect the oxygen concentration in the cell.

2P095 **細胞内の遊離ヘムの可視化に向けた融合タンパク質の開発**
Development of the FRET-based sensor protein for visualization of free heme *in vivo*

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Free heme, not taken in hemeproteins, is cytotoxic. The concentration of free heme regulates a variety of gene expression. However, the cellular labile heme pool has not been well characterized. Therefore, we developed the FRET-based sensor proteins. The heme-binding protein HrtR regulates the expression of the heme-efflux system in *Lactococcus lactis*. Thus, we fused HrtR mutant and fluorescent protein and measured FRET. The measurements of the fusion proteins demonstrated the decrease of FRET efficiency with the increase of the heme concentration *in vitro*. This result can be applied to develop the sensitive probe for the concentration of cellular free heme.

2P096 **紫外共鳴ラマン分光法によるインドールアミン 2,3-ジオキシシゲナーゼの三者複合体中間体モデルの研究****Ultraviolet resonance Raman study on a ternary complex intermediate model of indoleamine 2,3-dioxygenase**

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Indoleamine 2,3-dioxygenase (IDO) is a heme-enzyme and catalyzes insertion of two oxygen atoms into L-tryptophan (Trp), yielding N-formylkynurenine. Two oxygen atoms had been proposed to be inserted into Trp without cleaving the O-O bond. However, detection of a Fe=O species during reaction indicated that the O-O bond cleaved before insertion of the second oxygen atom.

The unusual reaction might be enabled by distinct heme-pocket environment including conformation of the IDO-bound substrate. In this study, we measured UV resonance Raman spectra of CN⁻-ligated and Trp-bound IDO as a model of IDO-O₂-Trp ternary complex, which is a key reaction intermediate. By utilizing UV resonance enhancement, we can selectively obtain vibrational spectra of the bound-substrate.