3P091 アクチン発現系の確立に向けて**Toward the establishment of an expression system for actin**

Masashi Mori¹, Yoshitaka Umetsu², Shinya Ohki² (¹Ishikawa Prefectural University, ²Japan Advanced Institute of Science and Technology)

Protein sample preparation is exactly the first step in every biophysical study. Curious proteins, however, are often difficult to be expressed by the popular systems including *E. coli* and *Pichia*. Actin has been recognized as one of such challenging proteins. So far, employment of various analytical methods requiring recombinant proteins is impeded for studying actin. To overcome this issue, we have been developing a new protein expression protocol with an inducible viral vector infection system in suspension-cultured plants cells. In the presentation, we will report overview of our original protein expression system and the preliminary results for actin.

3P092 インドールアミン 2,3 ジオキシゲナーゼの基質トリプトファンの検出-紫外共鳴ラマン分光法**Detection of the bound tryptophan in indoleamine 2,3-dioxygenase by UV resonance Raman spectroscopy**

Sachiko Yanagisawa¹, Masayuki Hara¹, Hiroshi Sugimoto², Yoshitsugu Shiro², Takashi Ogura¹ (¹Univ. of Hyogo, ²RIKEN Harima SPring-8 center)

A heme containing enzyme, indoleamine 2,3-dioxygenase (IDO) catalyses direct incorporation of two oxygen atoms into tryptophan (Trp) and its reaction mechanism is not fully understood due to the lack of structural information. Structural information of the bound substrate and intermediate products during the reaction is essential to understand the reaction mechanism. UV resonance Raman (RR) spectroscopy has been applied to selectively detect Trp in this study. We have successfully obtained RR spectra of the bound-substrate in IDO by the aid of its isotopomers. Some of the Raman bands exhibited intensity and/or frequency shift relative to those of free Trp. Here, we will present the UVR spectra of the bound-Trp and discuss its specific structure.

3P093 Interaction Between Heme and Heme-Cu Binuclear Center in Cytochrome *c* Oxidase

Miyuki Sakaguchi¹, Kyoko Shinzawa-Itoh², Shinya Yoshikawa², Takashi Ogura¹ (¹Department of Protein Vibrational Spectroscopy, Picobiology Institute, University of Hyogo, ²Department of Protein Crystal Growth Mechanism, Picobiology Institute, University of Hyogo)

The heme a_3 -Cu_B site and heme *a* in cytochrome *c* oxidase function as a O₂ reduction site and the driving element of H⁺-pump. Axial ligands of hemes *a* and a_3 are closely adjacent each other and they are on one α -helix, helix X. This structural feature is important for the coupling mechanism of the O₂ reduction reaction and the H⁺-pump.

In the resonance Raman spectra, the vibrational modes, which originate from the formyl substituent and the Fe-ligand bond of heme a_3 , showed frequency shifts upon the oxidation state change of heme *a*. This heme-heme interaction is most likely propagated through helix X. However, the frequency shifts were restored upon reduction of Cu_B. It suggests that the interaction could be regulated by the oxidation state of Cu_B.

3P094 チトクロム *c* 酸化酵素の酸素還元反応における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 the flow system with an oxygen lung aiming at IR measurement on the oxygen reduction reaction of cytochrome *c* oxidase**

Tatsuhito Nishiguchi, Masahide Hikita, Kyoko Shinzawa-Itoh, Shinya Yoshikawa, Satoru Nakashima, Takashi Ogura (*Grad. Sch. Lif. Sci., Univ. Hyogo*)

Although proton pump mechanisms of cytochrome *c* oxidase (CcO) have been studied vigorously, the detail is still unrevealed. Since IR spectra gives the information about the hydrogen bond related structure, it is extraordinary important to measure IR spectra during the reaction. Here we have developed a new flow system that enables to measure the time-resolved IR spectra on the oxygen reduction reaction of CcO in aqueous solution. The inserted artificial oxygen lung supplies oxygen, which is necessary for the reaction. To measure the IR spectra in aqueous solution, it is necessary to supply enough amount of flow rate in the extremely thick (50 μ m) water line. We could develop the flow system that could be used for time-resolved IR measurements.

3P095 シアン化物・アジ化物結合完全酸化型ウシ心筋チトクロム酸化酵素の構造解析**Structural analysis of bovine heart cytochrome *c* oxidase in the cyanide- and azide-bound fully oxidized states**

Kazumasa Muramoto¹, Masao Mochizuki¹, Naomine Yano¹, Tomoko Maeda¹, Kyoko Shinzawa-Itoh¹, Eiki Yamashita², Tomitake Tsukihara^{1,2}, Shinya Yoshikawa¹ (¹Grad. Sch. Life Sci., Univ. Hyogo, ²Inst. Protein Res., Osaka Univ.)

Cytochrome *c* oxidase (CcO) in the mitochondrial respiratory chain catalyzes the O₂ reduction coupled to proton pump. In the catalytic intermediate states, some of oxygen species are bound to the Fe_{a3}-Cu_B site. Cyanide (CN⁻) and azide (N₃⁻) ions, known as inhibitors, are bound to the Fe_{a3}-Cu_B site in the Fe³⁺ and Cu²⁺ state, which mimics oxidized form (O-form) intermediate.

In this study, we analyzed X-ray crystal structures of bovine heart CcO in the CN⁻- and N₃⁻-bound fully oxidized states. The refined structures indicate that CN⁻ bridges between Fe and Cu atoms and N₃⁻ shows two conformers, Fe- or Cu-bound form. Conformations of the Fe_{a3}-Cu_B site and proton transfer pathways are similar to those in the resting oxidized state, in which peroxide (O₂²⁻) ion is bound.

3P096 Sequencing bovine/human hybrid cytochrome *c* oxidase genes in HeLa cells to verify mutagenesis results disapproving D-path proton pumping

Ryohta Aminaka¹, Mai Itoh¹, Kunitoshi Shimokata², Yukie Katayama¹, Tomitake Tsukihara¹, Shinya Yoshikawa¹, Hideo Shimada¹ (¹Grad. Sch. Sci., Univ. Hyogo, ²WORLD INTEC CO., LTD.)

D-path proton pumping has been proposed from mutational studies on bacterial cytochrome *c* oxidase (CcO). Its structural similarity and amino acid conservation with bovine D-path imply D-path pumping in bovine CcO, contrary to our H-path proposal for bovine CcO. A bacterial D-path mutation abolishes proton pumping retaining O₂ reduction activity, supporting D-path model. However, as reported at previous meeting, the corresponding bovine mutation employing HeLa cell bovine/human hybrid CcO expression system does not change the activity, in contrast to the bacterial results. Here, for examination of the possible occurrence of back mutation of CcO in HeLa cells, we sequenced the CcO subunit genes or their cDNAs. No mutation other than the original one was identified.