ical characters. They bound to ligand peptide more strongly than wild-type, whereas they were less stable than wild-type. These results indicate that the library with primitive alphabets is available for creating functional proteins as well as the library with a full set of alphabets.

3P104 ヘムオキシゲナーゼによるベルドヘム開環機構 Ring Opening Mechanism of Verdoheme Catalyzed by Heme Oxygenase

Toshitaka Matsui¹, Masaki Unno², Masao Ikeda-Saito¹, (¹Institute of Multidisciplinary Research for Advanced Materials, Tohoku University: ²Frontier Research Center for Applied Atomic Sciences, Ibaraki University)

Heme oxygenase (HO) catalyzes degradation of heme to biliverdin, CO and a free iron to have a variety of physiological functions including heme catabolism and signal transduction. The HO reaction proceeds through three successive oxygenations in which the substrate heme and its degradation intermediates activate O₂ for their self-oxidation. The third oxygenation of the HO catalysis, ring opening of verdoheme to yield biliverdin, is considered as a major rate-determining step; however, this last oxygenation has been least understood in spite of its importance in regulating the HO activity. We have recently proposed a ring-opening mechanism involving Fe-OOH verdoheme and ferryl biliverdin intermediates on the basis of reaction analysis with various hydroperoxides. Crystal structural analysis of verdoheme-HO complexes in this study clearly reveals that the verdoheme-bound ligands can form hydrogen bond with a nearby water molecule. QM/MM calculations based on the crystal structures assure the feasibility of the proposed mechanism and highlight significance of the nearby water molecule in transferring the terminal OH group of Fe-OOH into an α -pyrrole carbon to cleave the porphyrin macrocycle. Generation of a low-spin ferryl species as a ring opening intermediate was revealed by Mossbauer analysis to support our mechanistic proposal.

3P105 ウシ心筋チトクロム酸化酵素 F 型中間体の X 線結晶構造解析 X-ray structural analysis of catalytic F intermediates in bovine cytochrome c oxidase

Ai Sasaki¹, Kazumasa Muramoto^{1,2}, maeda tomo¹, Kyoko Shinzawa-Itoh¹, Eiki Yamashita³, Tomitake Tsukihara^{2,3}, Shinya Yoshikawa^{1,2}, (¹Department of Life Science, University of Hyogo: ²Picobiology Institute, Department of Life Science, University of Hyogo: ³Institute for Protein Research, Osaka University)

Cytochrome *c* oxidase (CcO) catalyzes oxygen reduction coupled to the proton pump across the membrane. The oxygen reduction site consists of heme and copper atom. In the oxygen reduction cycle, several intermediate forms have been identified by visible absorption and resonance Raman spectroscopy. The oxygen-oxygen bond of oxygen molecule is broken on the formation of the P intermediate. In the next step, delivery of an electron and a proton to the catalytic site generates the F-form. X-ray structures of the F-form have not been determined yet. In this study, we prepared the crystals of bovine CcO containing F-form and performed X-ray structural analysis. It is known that reaction of the resting oxidized CcO with excess amount of hydrogen peroxide forms the F intermediate. The bovine CcO crystal in the resting oxidized state was treated by hydrogen peroxide, which caused the absorption change at 580 nm indicating the F intermediate formation. The resultant absorption spectrum indicated that although both the F-form and P-form exist in the crystal, the F-form is a dominant species. X-ray structural analysis showed that the electron density is located near Fe_{a3} and Cu_B. The result indicates that, one oxygen atom binds to Fe_{a3} and another oxygen atom binds to Cu_B in the F-form. Structure of the proton transfer pathway in the F-form is currently analyzed.

3P106 シアン化物結合混合原子価型ウシ心筋チトクロム酸化酵素の結晶の調製 A preparation of the crystalline cytochrome *c* oxidase in the cyanide bound mixed valence state from bovine heart.

Masao MOCHIZUKI, Kazumasa MURAMOTO, Kyoko SHINZAWA-ITOH, Shinya YOSHIKAWA, (Graduate School of Life Science, University of Hyogo)

Cytochrome *c* oxidase contains four redox-active metal centers, Fe_a, Fe_{a3}, Cu_A, Cu_B. For elucidation of the coupling mechanism of the electron transfer and the H⁺ pumping, analyses of the interactions between these metal centers is indispensable. Various respiratory inhibitors have been used for probing these interactions. Cyanide would be an excellent probe for examination of the effects of oxidation state of Cu_B on the ferric Fe_{a3}, since cyanide is the only reagent stabilizing Fe_{a3} in the ferric state. It is well known that Fe_{a3}³⁺-cyanide derivative is stable for 24 hours or longer in the presence of excess amount of dithionite. However, in the bovine heart

enzyme in the crystals, cyanide did not stabilize Fe_{a3}³⁺-cyanide completely as in the case in solution. Therefore, the stability of the Fe_{a3}³⁺-cyanide derivative of the enzyme in solution was reexamined for the present enzyme preparation. Upon addition of excess amount of dithionite, Fe_{a3}³⁺ was reduced within a few second while Fe_{a3}³⁺-cyanide derivative was reduced much slower. It took about 12 hours for the complete reduction. No structural basis for the difference in the stability is known. However, the integrity of the present preparation, providing single crystals diffracting high resolution X-rays, is obvious. The rate of Fe_{a3}³⁺ reduction much slower than that of Fe_a³⁺ strongly suggests that a mixed valence cyanide derivative in which only Fe_{a3} is in the oxidized state can be prepared. We are now searching the conditions for giving the derivative in the single crystals.

3P107 ヒトヘモグロビンの四次構造転移における芳香族アミノ酸残基の関与：近紫外円二色性による研究 Involvement of aromatic residues in the quaternary structure transition of human hemoglobin: A near-UV circular dichroism study

Masako Nagai, Kiyohiro Imai, Yukifumi Nagai, (Research Center for Micro-Nano Technology, Hosei University)

The negative circular dichroism (CD) band at 287nm of Hb A known as a T-state marker has been supposed to derive from the changes of Tyr and Trp residues at the $\alpha 1\beta 2$ subunit interface upon oxygen dissociation. To identify the aromatic residue responsible for the CD band, we have synthesized five recombinant Hbs in *E. coli* in which non aromatic residue is substituted for Tyr or Trp residue; rHb ($\alpha 14\text{Trp} \rightarrow \text{Leu}$), rHb($\beta 15\text{Trp} \rightarrow \text{Leu}$), rHb($\beta 37\text{Trp} \rightarrow \text{His}$), rHb($\alpha 42\text{Tyr} \rightarrow \text{Ser}$), and rHb($\beta 145\text{Tyr} \rightarrow \text{Thr}$). We examined the near-UV CD spectra of these rHbs and Hb Rouen($\alpha 140\text{Tyr} \rightarrow \text{His}$) in the oxy-form. The CD spectra of individual aromatic residue were extracted from the difference spectrum between Hb A and each mutant. Negative CD band appeared at 285nm for $\alpha 14\text{Trp}$ and $\beta 15\text{Trp}$, while positive CD bands at 280 and 287nm for $\beta 37\text{Trp}$. The $\alpha 140\text{Tyr}$ exhibited a positive CD band at 284nm, while $\alpha 42\text{Tyr}$ and $\beta 145\text{Tyr}$ showed a negative band at 274 and 286nm, respectively. The CD bands for $\beta 37\text{Trp}$, $\beta 145\text{Tyr}$, and $\alpha 140\text{Tyr}$ residues distinctly changed toward negative upon deoxygenation. On the other hand, the negative CD band of $\alpha 42\text{Tyr}$ changed became positive. While the CD bands for $\alpha 14\text{Trp}$ and $\beta 15\text{Trp}$ remained unchanged. We concluded that changes in CD bands arising from $\beta 37\text{Trp}$, $\alpha 140\text{Tyr}$, $\beta 145\text{Tyr}$ and $\alpha 42\text{Tyr}$ residues contributed to the appearance of the near-UV CD bands in different manners. To assign individual signals of CD band to each aromatic residue, we examined the effect of environments on CD spectra using model compounds of Tyr and Trp.

3P108 Spectroscopic analyses of heme-acceptor region of Bach2 protein

Murayama Kazutaka¹, Watamabe-Matsui Miki², Matsui Toshitaka³, Ikeda-Saito Masao³, Igarashi Kazuhiko², (¹Tohoku University, School of Biomedical Engineering: ²Tohoku University, School of Medicine: ³Tohoku University, Institute of Multidisciplinary Research for Advanced Materials)

The B cell-specific transcriptional repressor Bach2 possesses the basic region-leucine zipper (bZip) motif that mediates dimer formation with Maf oncoproteins and DNA binding. It has been found that heme inhibits the DNA binding activity of Bach2 in vitro and induces polyubiquitination and degradation of Bach2 in B cells. Furthermore, we found that heme bound directly to Bach2 in vitro through multiple Cys-Pro (CP) motifs by spectroscopic analyses. However, the regulation mechanism by heme is still unknown. Here, we are approaching to reveal this question with several spectroscopic measurements. Bach2(331-489) remained after limited proteolysis, suggesting that this region may be a domain-like form. While this region contains three of five CP motifs, secondary structure predictions by PSIPRED assigned no structural element for Bach2(331-489). Circular dichroism (CD) measurements in far-UV region indicated that Bach2(331-520) is almost random coil. Measurements in near-UV region indicated remarkable changes of spectrum in the presence of heme between Bach2(331-520) wild type and CP-mutant (cysteine to alanine at the CP motifs). This result suggested that the CP motifs are very important to accept heme. UV-Vis spectroscopic measurements also supported heme binding in this region. It is considered that Bach2(331-489) is not just randomly fluctuating but have a domain-like form and functions as a heme acceptor using the three CP motifs.

3P109 酸素化ヘモグロビン、ミオグロビンの酸化反応：pH 依存性と温度依存性 Oxidation reaction of oxyhemoglobin and oxymyoglobin: pH- and temperature-dependences

Wakasa Yuya¹, Iizuka Tetsutaro², Imai Kiyohiro², (¹Dept. of Frontier Biosci., Graduate Sch. of Engi., Hosei Univ.: ²Dept. of Frontier Biosci., Fac. of Biosci. and Appl. Chem., Hosei Univ.)