**211412 黄色ブドウ球菌鉄取り込み蛋白質 IsdH, IsdA, IsdC - 金属ボル
P33 フィリン間の結合および輸送機構解析**
**Analysis of iron and other metalloporphyrins recognition and
transport mechanism among IsdH, IsdA, IsdC from
Staphylococcus aureus**

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The development of vaccine and/or inhibitor for *Staphylococcus aureus* is demanded socially and medicinally because its rapid acquirement of resistance against antibiotic menaces human health. Iron-regulated surface determinants (Isds) are the best characterized heme (Fe-protoporphyrin; Fe-PPIX) transport system of *S. aureus*, distributed on the cell wall and in the cytoplasm, and are good targets to develop new antibiotics. We have studied the structures and the thermodynamics of the heme-binding to IsdH, IsdA, and IsdC (the 1st, 2nd and 3rd transporter of heme on cell wall, respectively). We found that (i) Ga-PPIX and Mn-PPIX can bind to the three Isd proteins like the natural ligand, Fe-PPIX, (ii) heme-binding site of IsdH undergoes a large conformational change upon binding to heme and (iii) the stepwise increase of the affinity for metalloporphyrins in the order of IsdH, IsdA, and IsdC enables heme to be transported to the cytoplasm without any energy sources. We are now investigating these points theoretically. Among them, to study the mechanism of the large conformational change, we performed a molecular dynamics simulation for the apo-form of IsdH. The heme-binding site showed a large fluctuation during the simulation and the conformational change in the holo-form was within the range of the fluctuation. This indicates that the conformational change upon binding to heme occurs by a population-shift mechanism.

**211424 リン脂質小胞に再構成したチトクロム c 酸化酵素の共鳴ラマン分光
法による研究**
**Resonance Raman Study on Cytochrome c Oxidase
Reconstituted in Phospholipid Vesicles**

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In mitochondria, protein complexes in the electron transport chain drive proton pumping upon electron transport. Proton pumping generate proton motive force ($\Delta\mu_{\text{H}}^+$). It consists of concentration gradient of proton (ΔpH) and electric potential ($\Delta\psi$). Electron transport rate depends on $\Delta\mu_{\text{H}}^+$. This phenomenon is called respiratory control. However, the effect of $\Delta\mu_{\text{H}}^+$ on the protein structure is not known.

We focus our attention on cytochrome *c* oxidase (CcO). CcO is the terminal enzyme of the respiratory chain in mitochondria. In the present study, we have prepared COV(cytochrome *c* oxidase reconstituted in phospholipid vesicles) and studied the structure of the heme selectively by resonance Raman (RR) spectroscopy. The reaction of CcO in COV is known to be affected by $\Delta\mu_{\text{H}}^+$. We have improved the preparation method of COV. As a result, the respiratory control ratio (RCR) of our COV is as high as 30. This COV could retain ΔpH and $\Delta\psi$ for a long time. The quality of RR spectra of the COV was comparable to that of solubilized CcO. We study the effect of $\Delta\mu_{\text{H}}^+$ on COV. We eliminated ΔpH or $\Delta\psi$ by FCCP or valinomycin and examined the effect on the RR spectra. The preliminary results suggested that the ΔpH altered the structure of CcO while the effect of $\Delta\psi$ was small.

211436 *cbb3*型チトクロム酸化酵素の発現条件の検討と精製
**Optimization of expression condition and purification of *cbb3*-
type cytochrome *c* oxidase**

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The terminal oxidases of the aerobic respiratory chains in mitochondria and bacteria catalyze the oxygen reduction coupled to proton pumping, which generates the proton-motive force. The terminal oxidases containing heme and copper at the catalytic site form the heme-Cu oxidase (HCO) super family. Although conformations of the catalytic sites are similar in HCOs, the proton transfer pathways are not conserved and the energy transducing efficiencies are different. To identify structurally important elements and clarify common

mechanism on the energy transduction, we aim to investigate *cbb3*-type cytochrome *c* oxidase from *Vibrio cholera* by X-ray crystallographic analysis. In this study, we examined culture condition for cells introduced the *cbb3* oxidase expression plasmid and partially purified *cbb3* oxidase using affinity chromatography. Expression level of *cbb3* oxidase was measured by absorption spectrum of the cell suspension and Western blotting. When cells were cultured under microaerobic condition, no significant expression was observed. When cells are cultured aerobically, expression levels gradually increased and saturated at 8 hours after induction. Purified *cbb3* oxidases showed characteristic absorption spectrum of hemes and oxygen consumption activity.

**211448 チトクロム c 酸化酵素のタンパク質構造ダイナミクスと反応機構：
共鳴ラマン分光法**
**Protein Dynamics and Reaction Mechanism of Cytochrome c
Oxidase: Resonance Raman Spectroscopy**

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Cytochrome *c* oxidase (CcO) is the terminal enzyme of the respiratory chain in mitochondria. It reduces dioxygen to water and translocates protons from the negative to the positive sides of the membrane. Helix X (α -helix) near the two hemes exhibits conformational change upon redox-change and ligand binding of heme a_3 , and contains His376, which is the axial ligand to hemes a_3 . The conformational change in Helix X between the main chain C=O of Ser382 and Val380 is suggested to function as a gate in the proton transport pathway to prevent proton back-flow.

Time-resolved resonance Raman (TR³) results showed that there were two distinct phases in the time profile of the $\nu_{\text{Fe-His}}$ after CO-photolysis. The $\nu_{\text{Fe-His}}$ frequency was constant at 220 cm^{-1} between 10 and 100 ns, and underwent frequency decrease exponentially with time to 215 cm^{-1} with a rate constant of $1.3 \times 10^6 \text{ s}^{-1}$. Since the main chain C=O of His376 is hydrogen-bonded to the main chain NH of Val380, the conformational change in Helix X is likely to alter the $\nu_{\text{Fe-His}}$ frequency, which thus reflect the state of the gate.

A highest turnover number of the present CcO preparation under the same solution conditions of those in Raman measurements was 310 s^{-1} , which means that 1 reaction cycle takes 3 ms. The results suggest that the timescale of proton gate is fast enough to prevent proton back-flow.

Several protein dynamics observed by TR³ spectroscopy will be discussed in the light of time scale of enzyme turnover.

**211512 A computational study of functional role of axial methionine
ligand of Cu_A site in cytochrome c oxidase**

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Cytochrome *c* oxidase (CcO) reduces an oxygen molecule by coupling to the proton pumping, thereby generating the gradient of the proton concentration between the matrix and the intermembrane space of mitochondria or the periplasmic space of bacteria. The Cu_A site of CcO receives electrons from cytochrome *c*, providing the electrons with heme *a*. Thus, the Cu_A site acts as the "gate" of the subsequent cooperative reactions in CcO.

The replacement of the axial Met ligand with leucine (M207L for bovine CcO) largely induced the changes of the redox potentials in various species. In fact, for *R. sphaeroides* CcO, the redox potential of the M207L mutant largely increases (118 mV); but, for *T. thermophilus* ba₃ oxidase and the engineered azurin, which is a reconstructed azurin possessing a similar Cu-coordination to the Cu_A site, the influence of the mutation is not significant (53 mV and 16 mV, respectively). Thus, the functional role of this Met residue was not clear.

To unravel this issue, we investigated the geometrical and electronic structures of the Cu_A site in various oxidases, exploiting structural modeling and hybrid *ab initio* quantum mechanics / molecular mechanics calculations. As a result of the analysis, we found that the presence/absence of the inserted structural element close to the Cu_A site is relevant to the redox potentials, whereas the naive effect of the Met residue is not significant. In the session, we discuss the detailed differences of the electronic structure of the Cu_A site of the WT and the mutant enzymes.

**211524 CN⁻光解離に伴うチトクロム c 酸化酵素の構造ダイナミクスの共鳴
ラマン分光法による追跡**
**Protein Dynamics of Cytochrome c Oxidase upon CN⁻
Photolysis as Studied with Resonance Raman Spectroscopy**