3P088 *Tetrahymena* 短縮型ヘモグロビンの酸素安定性と結晶構造解析
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Oxygen stability and crystal structure of *Tetrahymena* truncated hemoglobin
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Truncated hemoglobins (trHbs) are distributed from bacteria to unicellular eukaryotes and play a role in oxygen transport and nitric oxide detoxification. It has been known that trHbs exist in the ciliates, *Tetrahymena*, but its function and structure was unknown. To understand the function in relation to stability of bound oxygen and its structure, we obtained the autoxidation rate of *T. pyriformis* trHb, and solved the crystal structure. The autoxidation rate of *T. pyriformis* trHb was determined to $3.8 \times 10^{-3} \text{ h}^{-1}$ at 25 °C, pH 7.2. This value is comparable to that of sperm whale myoglobin ($5.0 \times 10^{-3} \text{ h}^{-1}$) but is 10 times slower than that of ciliates, *Paramecium caudatum* trHb ($3.1 \times 10^{-2} \text{ h}^{-1}$). The three dimensional structure of oxygenated *T. pyriformis* trHb was solved at 1.73 Å resolution. Tyr25 and Gln46 were hydrogen-bonded to heme bound dioxygen molecule. Tyr25 donated hydrogen bond to the terminal oxygen atom, whereas Gln46 hydrogen-bonded to proximal, iron-bound, oxygen atom. Furthermore, Tyr25 was hydrogen bonded to Gln46 and Gln50 residues. To confirm the role of Tyr25, we made Y25F mutant. The autoxidation rate of Y25F mutant was 2 orders of magnitude faster than that of wild-type protein. These data suggest that hydrogen bonding from Tyr25 to dioxygen and the other residues regulate the oxygen stability of *T. pyriformis* trHb.

3P090 クライオ電子顕微鏡法によるヘモオキシゲナーゼ-1・シトクロム P450 還元酵素複合体の結合様式の解明
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Determination of structure of heme oxygenase-1 and cytochrome P450 reductase complex by electron cryo-microscopy
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Heme oxygenase-1(HO-1) is a key enzyme in heme degradation system and catalyzes degradation of heme using electrons donated by NADPH-cytochrome P450 reductase (CPR). However, the CPR/HO-1 complex and the mechanism of electron transfer have been still unclear. Thus we revealed the three-dimensional structure of the CPR/HO-1 complex by electron cryo-microscopy. RatHO-1 fused by GST was expressed in *E. coli* to be purified and then the frozen-hydrated one was observed by electron cryo-microscopy. We compared each molecule recognized as CPR/GST-HO-1 complexes on obtained electron micrographs with atomic models from X-ray crystallography and a simulated model of their complex. Furthermore we determined the projection angle of each molecular image and so obtained a three-dimensional image of the complex. Comparing each molecular image obtained by cryo-electron microscopy with the simulation model of the CPR/HO-1 complex indicated that CPR/HO-1 complex should be similar in structure to the simulation model. Further the three-dimensional model by single particle analysis showed that the binding angle of HO-1 against CPR differs from the simulation model a little. By three-dimensional modeling, we suggest that HO-1 binds to CPR as close approach to FMN domain and it makes efficient electron transfer possible.

3P089 ヘム制御真核生物 eIF2 α キナーゼ、HRI、のヘム結合部位
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Site-directed mutagenesis study to identify the heme binding site in a heme-regulated eukaryotic initiation factor 2 α kinase, HRI
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Under conditions of stress, such as nutrition deficiency, accumulation of unfolded protein, virus infection and shortage of heme iron, cells initiate protection mechanisms by terminating protein synthesis via Ser51 phosphorylation of the α subunit of eukaryotic initiation factors 2 (eIF2 α) by eIF2 α kinases. In healthy red cells, the ratio of the heme iron to globin protein is 1:1. Upon depletion of the heme iron, the system attempts to terminate globin synthesis and remedy the heme-to-protein ratio imbalance. HRI senses low heme iron concentrations at the putative heme binding site. It is proposed that His in the N-terminal domain and Cys in the C-terminal domain are axial ligands of the Fe(III) heme. Thiolate coordination to the Fe(II) heme is relatively weak, and ligand switching from Cys to His often occurs upon heme iron reduction. Also it was found that the heme dissociation rate constant from the apo HRI protein is markedly fast compared with those of myoglobin and hemoglobin, HRI may use these properties to regulate the catalytic function in response to the heme concentration. However, the molecular mechanism of heme sensing and the axial ligands in the putative heme binding sites of HRI remain to be established. We generated single amino acid and double amino acid mutants of the conserved His and Cys residues of HRI, and examined their heme binding characteristics, optical absorption spectra, and catalytic inhibition by heme. Based on the experimental findings, the heme sensing mechanism of HRI is further discussed.

3P091 電子分光クライオ電子顕微鏡法による呼吸鎖シトクロム c 酸化酵素の三次元構造決定
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Determination of the three-dimensional structure of respiratory chain cytochrome c oxidase by electron cryo-microscopy
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Respiratory chain cytochrome c oxidase is a membrane protein related to the electron transport chain. This protein accepts an electron from cytochrome c, the last electron carrier, working at the end of the respiratory chain. The electron is sent to O₂ as the last electron acceptor. However, the mechanism how the electron can transfer from bound cytochrome c is still unclear. We here report how bo3 type cytochrome c oxidase from *Bacillus thermodenitrificans*, whose 3D structure has not been reported yet, binds a cytochrome c. The frozen-hydrated specimens were observed under the conditions with or without cytochrome c by electron cryo-microscopy, respectively. As a result, the difference between substrate-bound and substrate-unbound oxidase was identified on 2D averaged maps after classification and averaging. The orientation of each molecule from the electron micrographs was determined by comparison with the atomic models of the other species of oxidase and then the 3D maps were reconstructed under each condition. When the obtained three-dimensional images were compared with the atomic structures of each subunit, the density corresponding to the binding site of cytochrom c that had been even suggested would be identified as the region projecting from the oxidase but another protruding density also was found. The rough sites of cytochrome c could be identified but the detailed structure and its precise binding sites will be determined by refinement and their resolutional improvement.