2P112 逆分子系統解析法による円口類と硬骨魚類の祖先型ヘモグロビン
Ancestral hemoglobins of Cyclostomata and Osteichthyes as studied by reverse-molecular phylogenetic analyses

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The large majority of fish which is classified into Osteichthyes of jawed vertebrates, generally possesses the tetrameric hemoglobin (Hb) composed of α and β globins as human Hb. In contrast, the oldest extant jawless vertebrates, hagfish and lamprey which are classified into Cyclostomata, have the monomeric Hb undergoing a quaternary structural transition: monomer in oxy-form changes to dimer or tetramer in deoxy-form. To understand how the vertebrate Hbs have acquired the tetrameric structure and functions from primary monomeric globins through the evolution, we deduced the amino acid sequences of the ancestral Hbs of Cyclostomes and Osteichthyes by the reverse-molecular phylogenetic analyses using more than 1,200 sequences of the fish Hbs registered in the nucleotide and protein databases. These deduced ancestral Hbs were synthesized by using *E. coli* expression systems. The maximum-likelihood phylogenetic analyses made it clear that cyclostomate Hbs will be more closely related to cytoglobin (Cgb) rather than to myoglobin and Hb of the jawed vertebrates, suggesting that the jawless vertebrates may have adopted the Cgb as the oxygen carrier in the red cell by way of a different evolution. This is the first report on the ancestral Hbs of the jawed and jawless vertebrates of fish.

2P113 Characterization of hisidine-tagged cytochrome P450cam and its synthesis in a cell-free system

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Cytochrome P450cam (P450cam), a soluble hemeprotein of 45 KDa, catalyses monooxygenation of D-camphor to yield 5-*exo*-hydroxycamphor. Infrared spectral analysis of the amino acid residues at the functional site, which is essential for understanding the catalytic mechanism, require their site-specific isotope labeling, which is achievable only by cell-free protein synthesis, to distinguish their infrared spectra overlapped with those of the other amino acid residues with the same functional groups.

Here, to facilitate the purification of P450cam, we constructed an expression vector for P450cam with C-terminal histidine tag. The His-tagged version of P450cam expressed in *E. coli* cells was highly purified by a single step of a Ni²⁺-NTA column chromatography in contrast to the previous procedures including ammonium sulfate fractionation, and ion exchange, gel filtration and blue-Sepharose column chromatographies. The absorption spectra, the enzymatic activity, and the X-ray structure were indistinguishable from those of the wild type enzyme except for the tag. Hence, the enzyme samples could be available for further experiments. We synthesized 0.5-1.0 mg enzyme samples in 1 ml *E. coli* cell-free system supplemented with DnaK and GroE. Eluate from a Ni²⁺-NTA column contained an inactive form (P420), which was removed by a phenyl-Sepharose column chromatography, yielding the purified samples of approximately 180 μ g. The present results indicate establishment of an efficient cell-free synthesis and purification system of P450cam.

2P114 Cys-93 β を *N*-ethylmaleimide で修飾したヘモグロビンは二量体への解離度が低い
Hemoglobin with Cys-93 β treated with *N*-ethylmaleimide shows smaller dissociation into dimers

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Vertebrate hemoglobin is in an equilibrium between the $\alpha_2\beta_2$ tetramer and the $\alpha\beta$ dimers. This equilibrium is shifted toward dimers upon dilution of the Hb solution and also upon oxygenation when the several intersubunit salt-bridges formed in deoxyHb are ruptured. The chemical modification of the sulfhydryl group of cysteine-93(F9) β with *N*-ethylmaleimide (NEM) causes the breakage of the salt-bridge in deoxyHb between the C-terminal histidine imidazole group and the γ -carboxyl group of aspartate-94(FG4) of the same β chain. This breakage will, in turn, reduce the stability of another salt-bridge between the β chain C-terminal carboxyl group and the ϵ -amino group of lysine-40(C5) of the α chain of the $\alpha_1\beta_2$ pair. Thus, it is expected that this modification will enhance the dissociation of Hb tetramer into dimers. Inao et al. (2009) measured tetramer-to-dimer dissociation constants for native Hb and NEM-treated Hb by analyzing their oxygen equilibrium curves at different protein concentrations. The results indicated that the modification with NEM

does not enhance the dissociation into dimers. We further examined this result with molecular sieve column and by measuring dimer-to-tetramer association rate. These experiments supported our previous observation that NEM-treated Hb is less dissociated to dimers than native Hb.

2P115 MD simulation study on the atomic-level structure of ABCC7 (CFTR)

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Cystic fibrosis transmembrane conductance regulator (CFTR) acts as a chloride ion channel, and is involved in cystic fibrosis, which causes abnormal exocrine glands. According to a homology modeling structure (Mornon et al. Cell. Mol. Life Sci. (2009) 66, 3469), CFTR has two transmembrane domains (TMDs), two nucleotide binding domains (NBDs), and a regulatory domain. In the present study, we performed MD simulations to obtain the atomic-level structure of CFTR in the ATP-bound and -unbound states, which provides invaluable information about the role of ATP in the functional mechanism of CFTR. In the homology modeling structure, the TMDs take an outward-facing conformation, and the NBDs are dimerized to form two ATP binding pockets (ABP). We assumed two ATP binding states: 1) two ABPs were occupied simultaneously by two ATP molecules, and 2) they were vacant. One molecule of such a protein was put into a hydrated lipid bilayer membrane and MD simulation was started. After 50 ns MD, a drastic conformational change was observed in the case 1): a salt-bridge was formed between Lys892 and Asp110, so that the outward-facing conformation was considerably closed. Such a conformational change was not observed in the case 2). To study the role of ATP in more detail, we removed two ATP molecules from the ABPs immediately after 30 ns MD of the case 1), and continued to perform MD simulation. We are now investigating the protein dynamics and structure of such a system.

2P116 Three-dimensional Solid-state NMR study of *Anabaena* Sensory Rhodopsin in the lipid environment -Chemical Shift Assignments-

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Microbial rhodopsins are photoactive membrane proteins triggered by retinal photoisomerization, expressing ion-pump and photosensory functions in archaea, bacteria and eukaryote. We have demonstrated the structural insight of marine Proteorhodopsin (PR) by multidimensional chemical shift correlation experiments [1,2].

Anabaena Sensory Rhodopsin (ASR) from cyanobacteria may be photo-receptor for chromatic adaptation and interacts with soluble transducer (ASRT). It is so important to investigate structure and protein-protein interaction mechanism to understand its function. Here, we performed 3D sequential chemical shift assignments of U-¹³C, ¹⁵N-labelled ASR sample in the lipid environment by solid-state NMR. We obtained assignments for 169 residues, 873 atoms and structural insight on the basis of chemical shift. In this meeting, we will show the ¹³C γ chemical shifts of proton acceptor Asp217 in the cytoplasmic side [3] compared with that of Asp75, Asp85 in BR or Asp97 in PR, and BC loop conformation which missing from electron density in crystal structure.

[1] L. Shi et al. (2009) *BBA* 1788 2563-2574. [2] L. Shi et al. (2009) *JMB* 386 1078-1093. [3] L. Shi et al. (2006) *JMB* 358 686-700.

2P117 細菌機械受容チャネル MscL の開口には水分子とゲート部位の水素結合形成が必要である
Channel Opening of the Bacterial Mechanosensitive Channel MscL Requires Hydrogen Bond Formation between the Gate and Water Molecules

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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer (TM1, TM2) α -helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. To address this question, molecular dynamics (MD) studies have been performed, however, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. Thus we performed MD simulations for the opening of MscL embedded in the lipid bilayer. Among amino acids in TM2 facing the bilayer, Phe78 showed exceptionally strong interaction with lipids. Upon membrane stretch, Phe78 was dragged by lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. Neighbor-