in terms of the water entropy. The order of the measure is in accord with that of the thermal stability: PA_{c551} < PH_{c552} < HT_{c552} < AA_{c555}. However, AA_{c555} has the lowest packing efficiency of heme and the holo-like structure of apo polypeptide, which is unfavorable for the water entropy. The highest stability of AA_{c555} is ascribed to the highest efficiency of side-chain packing of the apo polypeptide itself. For the apo states, AA_{c555} has much higher thermal stability than the other three. Overall, the theoretical results are quite consistent with the experimental observations [2,3]. We thank K. Oda for his contribution to the present study.

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1P112 表面プラズモン共鳴法を用いた nNOS アイソフォームへのカルモジュリン結合の解析
Analysis of calmodulin binding to nNOS isoforms using surface plasmon resonance

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NOS consists of two domains; an oxygenase domain, containing heme, tetrahydrobiopterin, and the arginine-binding sites, and a reductase domain, containing the FMN, FAD and NADPH-binding sites. These two domains are connected by a calmodulin (CaM)-binding site and the electron transfer required for NO formation is regulated by the reversible binding of Ca²⁺/CaM. nNOS reductase domain contains an autoinhibitory domain (AH), which is proposed to compete with CaM binding site for CaM binding by analysis of catalytic activities. NO formation is also regulated by phosphorylation at several Ser sites located within AH domain. To examine the role of the AH domain in CaM binding, we prepared the purified nNOSalpha, nNOSdelta40 and nNOSmyu by using *E. coli* expression systems. nNOSalpha is a common neuronal isoform and nNOSdelta40 is an AH deletion mutant. nNOSmyu is an isoform of nNOS mainly expressed in muscle and has an extra-34-amino-acids in AH domain. We have analyzed interactions between each of nNOS isoforms and CaM using surface plasmon resonance. Kinetic data indicated that the CaM binding affinity is decreased in the order of nNOSmyu, nNOS-alpha > nNOSdelta40. These results suggest that AH domain directly affects the interaction with CaM. Next, we prepared phosphomimetic mutants in that Ser-847, Ser-857 or Ser-858 located in AH domain was substituted with Asp. Analysis of interaction between each of these mutants and CaM is now undertaken. We will discuss the effects of AH domain on CaM binding.

1P113 水チャネルアクリポリン-4 細胞内側構造と機能連関
Involvement of cytoplasmic construction with aquaporin-4 functions

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Phosphorylation of Ser 180 in cytoplasmic loop D has been shown to reduce the water permeability of aquaporin-4 (AQP4), the predominant water channel in brain. Nevertheless, the structure of a mutant (AQP4M23S180D) potentially mimicking a phosphorylation of Ser180 to Asp of the AQP4M23 revealed to have similar structural properties with that of the wild type water channel. Partially ordered structure tends to be unresolved at high resolution but could be sometimes discriminated in three dimensional structure reconstructed from images including lower resolution data. Therefore, together with structure of AQP4M23S180D analyzed at 2.8 Å by diffraction patterns, we analyzed the structure at 10 Å resolutions from image data. The main features of 10 Å map are consistent with the atomic model at 2.8 Å resolution showing no obstacles near the pore entrance. Additionally, measurements of water conductance for the mutant in vitro shows as high conductance as that for the wild type, suggesting that the negative charge mimicking the phosphorylation state in Loop D neither induce a significant conformational change of the channel. In this study, the 10 Å map clearly shows a cytoplasmic density in between four adjacent tetramers that most likely represents the association of four N termini, supporting that the critical role of the N terminus of AQP4 in the stabilization of orthogonal arrays and also their interference by lipid modification on cysteine residues of the longer N terminus isoform.

1P114 Sec トランスロコンと共に機能する SecDF 膜タンパク質の構造
1YA1000 Crystal structure of SecDF, a Sec translocon-associated membrane protein

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Transmembrane protein translocation in bacteria is driven by dynamic interplays between the protein-conducting SecYEG channel (translocon) and the SecA ATPase. A membrane-integrated Sec component, SecDF, associates with SecYEG and facilitates the completion of protein export, but its structure and exact function have remained elusive. Here, we report the 3.5 Å-resolution crystal structure of SecDF from *Thermus thermophilus* and higher resolution structures of its periplasmic domains. SecDF consists of a pseudo-symmetrical 12-helix transmembrane domain and two protruding periplasmic domains with novel head-base architectures. The head subdomain of the first periplasmic domain assumes at least two different orientations relative to the base. Crosslinking experiments demonstrated that this conformational transition of the periplasmic domain is crucial for the SecDF function. Intriguingly, the geometrical arrangements of the transmembrane helices of SecDF are virtually identical to those of AcrB, an RND family multi drug-proton antiporter. Several charged residues at the transmembrane SecD/SecF-interface are functionally important, including an evolutionarily conserved aspartate, whose counterpart in AcrB is involved in the conduction of protons. We propose that SecDF functions as a dynamic, membrane-integrated chaperone, which is possibly powered by the proton-motive force, to actively complete SecYEG-mediated protein translocation.

1P115 高親和性 IgE 受容体 β 鎖変異体 (D234A) の構造解析
Secondary Structure and Conformation Change in mutated FcεRI β Chain (D234A) Investigated by Circular Dichroism Spectroscopy

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The β chain of the high affinity IgE Fc receptor (FcεRI) acts during an allergic reaction as a signal amplifier in mast cells. Previously, we revealed the biological functions mutated β chain (D234A) in mast cell activation upon FcεRI engagement and demonstrated that D234A severely impaired FcεRI-mediated degranulation, cytokine secretion (IL-6, IL13), leukotriene C4 secretion. This time, we investigated the structure of the C-terminal region of FcεRI β chain wild type (WT) and D234A. The far-UV CD spectra of WT and D234A are of an α-helical protein and D234A does not have any loss or collapse of α-helical content. Near-UV CD spectroscopy study that a conformational change in the local environment around tyrosine has not occurred for D234A compared with WT. We have revealed the thermal unfolding of the secondary-structural elements of FcεRI β chain WT and D234A. Free energy change (ΔG) of WT and D234A is 67.1 and 50.4 kJ/mol, respectively. Change in ΔG suggests that D234A do not affect secondary and tertiary structure of the protein, however, may weaken the strength of hydrogen bonding of the protein. Our findings suggest that D234A is crucial for normal degranulation and cytokine secretion in mast cell, however, secondary structure and conformation change in deletion mutant do not occur at ambient temperature.

1P116 Correlation between tertiary structure and solubility in Dodecyl-β-D-maltoside on membrane protein bacteriorhodopsin

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A seven transmembrane protein bacteriorhodopsin (bR) exists on the biological membrane of *Halobacterium salinarum*, and forms two-dimensional lattice structure called purple membrane (PM) by specific interaction between bR molecules or bR and lipids. bR that functions as light-driven proton pump shows a large shift of λ_{max} and a subtle tertiary structural change accompanied by the binding/release of a retinal and the photocycle after photon absorption. In this study, we investigated the correlation between the structural change and the solubility in a non-ionic detergent, Dodecyl-β-D-maltoside (DDM) on bR. When PM was treated by 20 mM-DDM at 25 °C for 24 h, only about 20 % of bR was solubilized. Contrary to this, all protein molecules were solubilized from the completely photo-bleached, that is, retinal-released membrane by hydroxylamine. The resistance of the membrane to DDM was increased reversibly by re-addition of the retinal. In addition, the plots of solubilization ratio of the protein by DDM versus bleaching ratio of PM were well fitted by a simple exponential curve, indicating that the ratio of retinal-released protein in PM can be calculated from the solubilization ratio. Interestingly, the solubility of bR in PM was also increased under light irradiated condition, in proportion to the light intensity. These results suggest that the change of the solubility of bR in DDM is attributed to the tertiary structural change accompanied by the ligand binding/release or the photocycle.