2P-032 [NiFe] ヒドロゲナーゼの FT-IR による研究

FT-IR study of the active site of [NiFe] hydrogenase

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[NiFe] hydrogenase has a unique metal-complex system as an active site in the large subunit. Two metal atoms, Ni and Fe, are supported by four cysteine sulfur atoms. Two of sulfur ligands form a bridge between Fe and Ni. Fe has an additional three non-protein diatomic ligands, and a third bridging ligand in the inactive-oxidized form. We have determined the crystal structures of [NiFe] hydrogenase from *D. v. Miyazaki* F in the various oxidation states, such as as-purified (mixture of Ni-A and Ni-B), Ni-A (inactive-unready-oxidized), Ni-B (inactive-ready-oxidized), Ni-C (active-reduced), Ni-L (light-irradiated) and CO-inhibited (CO-bound) forms at high resolution. These crystal structures have contributed to understanding the activation mechanism of the active site from the inactive-oxidized to active-reduced states. Whereas, since the catalytic reaction is considered to be controlled by the non-protein ligands of the Fe atom, we have started the spectroscopic studies of the active site by using the FT-IR method. [NiFe] hydrogenase in the Ni-B state has the bands at 1955, 2081 and 2090 cm⁻¹ assigned as non-protein ligands of CO and two CN, respectively. It has been known that the enzyme in the Ni-C state can beconverted to the Ni-L state by illumination of the strong white light. We have recently found that the IR-band at 1955 cm⁻¹ in the Ni-B state is upshifted to 1971 cm⁻¹ by illumination of Ar⁺ laser (476.5 nm). The comparison of the ligand structures between the Ni-L and this new light induced state will be discussed.

2P-033 小胞体に内在するジスルフィド結合還元酵素 ERdj5 の構造・機能解析

Structural and functional analyses of an ER-resident protein disulfide reductase, ERdj5

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In eukaryotic cells, the endoplasmic reticulum (ER) is the space where most of membrane or secretory proteins fold, assemble and form disulfide bonds. While the ER has a system that promotes correct folding of newly synthesized polypeptides, quality control mechanisms operate in this compartment to properly deal with terminally misfolded proteins. One of the ER quality control mechanisms, ER-associated degradation (ERAD), requires some ER resident proteins that recognize, reduce and retrotranslocate the terminally misfolded proteins. Recently, ERdj5 has been identified as an EDEM-binding protein that reduces specifically incorrect disulfide bonds of misfolded proteins in a relatively oxidative environment of the ER. ERdj5 is predicted to have a J-domain to bind a molecular chaperon, BiP, and four tandem thioredoxin-like domains. We here succeeded in solving crystal structure of a redox-active domain of ERdj5, Trx4, at a resolution of 2.0 Å. Besides, the redox potential, insulin reduction activity and *in vivo* ERAD activity of this domain have been investigated in details. Now, crystal structure analysis of full-length ERdj5 is in progress. On the basis of these experimental data, we will report on our new insights into molecular mechanism of ERdj5.

2P-034 タンパク質から切り出されてきた多様な 50 残基長セグメントに見られる構造相関ネットワークの特徴

Two types universality of a fold universe of 50-residue segments.

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To study a fold universe of 50 residue protein segments, in this study, we took the 1803 domains from the SCOP (α , β , $\alpha+\beta$, and α/β classes), and cut them into the 50-residue segments, which produced 186,821 segments. We classified the segments into number (Kc) of clusters, and embedded the clusters into a three-dimensional conformational subspace based on inter-residue contact patterns in the segments. We examined diversified values for Kc to construct the subspace. In a wide range of Kc ($1000 \leq Kc \leq 5000$), the conformational subspace was partitioned into four fold regions (mainly- α , mainly- β , $\alpha\beta$, and random-structure regions), and the relative positioning of the four regions was conserved in the subspace, where the mainly- α , mainly- β , and α/β regions surrounded the random-structure regions. Next, we constructed a network by linking clusters that had a structural similarity to each other in the full dimensional space. Then the network was characterized by a quantity "community", where clusters were tightly linked within each community but weakly done between different communities. The number of major communities was confined between 30 and 40 in the range of $1000 \leq Kc \leq 5000$. Each community was involved in one of the four fold regions in the space partitioning. Furthermore, most of the communities were conserved in the range of Kc. We conclude that the conformational space has two types of universality: the space-partitioning universality and the network-partitioning universality. This knowledge at the segment level is a new finding.

2P-035 口蹄疫ウイルス外殻蛋白質のシミュレーション:FMDV ループ構造多様性の解析

Dynamics of the GH loop on the surface of the foot-and-mouth disease virus capsid

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The major immunogenic site of the foot-and-mouth disease virus (FMDV) capsid is the loop between the G and H strands of the VP1 protein (FMDV loop). The FMDV loop is also important for attachment to host cells through the integrin mediated recognition process by the Arg-Gly-Asp (RGD) tripeptide in the midst of the loop. The FMDV loop is so flexible that its X-ray structure has not been determined except for the reduced capsid of the serotype O, in which the loop lies at the "down position" on a minor surface depression, directed toward the 3-fold axis. As simulation is the most powerful method to explore dynamics that can not be clarified by experimental approaches, we performed an MD simulation of the unreduced FMDV capsid under rotational symmetry boundary condition for 15 ns, starting from the reduced capsid coordinates. On breaking the disulfide bond at the base of the FMDV loop by the molecular mutation method, the FMDV loop rose from the capsid surface, began showing high mobility like a tentacle of sea anemone. However, the conformation of the RGD tripeptide was rather rigid, conserving open turn conformation. The structure of the residues at the base of the FMDV loop was compared with the X-ray analysis of the unreduced capsid, indicating that the simulation results are consistent with the hypothesis of the "up position". The conformation of the RGD tripeptide was also analyzed regarding the host tropism of FMDV through difference of binding specificity between the integrin subtypes of $\alpha IIB\beta 3$ and $\alpha v\beta 3$.

2P-036 分子動力学計算による ABC トランスポーター Sav1866 の薬剤輸送機構の研究

Molecular dynamics simulation reveals the drug transport mechanism of ABC transporter Sav1866

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The ATP-binding cassette (ABC) transporters are integral membrane proteins which couple the energy of ATP hydrolysis with the translocation of chemically diverse substrates across the lipid bilayers of cellular membranes. ABC transporters are generally classed as either exporters or importers. For example, human ABC exporters, such as ABCB1 and ABCC1, contribute to multidrug resistance of cancer cells through the extrusion of cytotoxic compounds. Despite the various studies, however, a detailed transport system has remained elusive. Sav1866, a *S. aureus* homolog of human ABCB1, is one of the structure-determined ABC transporters. In this study, we performed MD simulation for Sav1866 to understand its drug transport mechanism. The initial structure of Sav1866 was taken from ADP-bound X-ray structure (PDB code: 2HYD) and embedded in a hydrated lipid bilayer membrane. From this simulation, we found the following characteristic features. First, the principal component analysis revealed the occurrence of an opening/closing motion in the transmembrane domain that forms a pathway for transporting a trapped drug. Second, the helix bundle of the TMD was more closely packed in the final structure than that in the initial X-ray structure, in which the TMD takes an outward-facing conformation with an extrusion pocket exposed to the external medium. These findings suggest that Sav1866 with ADPs is transforming its structure from outward-facing into inward-facing so that the drug binding site is accessible from the cell interior.

2P-037 3 α -hydroxysteroid dehydrogenase の補因子結合に伴う構造変化の構造および生化学的解析Structural and biochemical analysis for the cofactor-induced conformational change of 3 α -hydroxysteroid dehydrogenase

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Bacterial 3 α -hydroxysteroid dehydrogenase (3 α HSD) is a member of the short-chain dehydrogenase/reductase (SDR) superfamily. We determined the crystal structure of 3 α HSD from *Pseudomonas* sp. B-0831 (Ps3 α HSD) in complex with NADH, showing that the enzyme exists as a heterodimer composed of apo- and holo-subunits. Comparing the holo-subunit with the apo-subunit, it was found that the NADH binding induces a significant conformational change, the loop-helix transition, in the substrate binding site, which would be correlated with the catalytic function. The induction of α -helices in solution by the nucleotide cofactor binding was also confirmed by the CD experiment, and also supported by the molecular dynamics (MD) simulation. In this study, we focused on the key residues for the conformational change of Ps3 α HSD, based on the results of crystal structure and MD simulation, and prepared the five mutant proteins, T75A, Q148R, T188A, L190A, and Y200A. For the mutant preparation, we first cloned and overexpressed them in *E. coli* with N-terminal His-tag. The respective far-UV CD spectra of the five mutants purified by an affinity chromatography were similar to that of the wild-type. The catalytic activities of the mutants, T75A, Q148R, and L190A, for cholic acid as a substrate were similar to that of wild-type,