The flagellum is a motile organelle composed of the basal body rings and the tubular axial structure. The axial component proteins synthesized in the cytoplasm are transferred into the central channel of the flagellum by the flagellar type III protein export apparatus for self-assembly at the growing end. The apparatus is composed of six transmembrane proteins (FlhA, FlhB, FlhO, FlhP, FlhQ, FlhR) and three soluble components (FlhI, FlhJ, FlhK). FlhJ is an essential component for protein export. Although FlhJ is thought to be a general chaperone, its function is still unclear. We determined the crystal structure of FlhJ at 2.1 Å resolution. FlhJ has a remarkable structural similarity to the γ subunit of F₀F₁-ATP synthase. Interestingly, it has already been shown that the FlhI structure closely resembles those of the α/β subunits of F₀F₁-ATP synthase and that FlhI forms a hexameric ring structure. These similarities suggest that FlhI and FlhJ may form a complex just like F₁-ATPase. We therefore analyzed the interaction between FlhJ and FlhI by electron microscopy and biochemical techniques. FlhJ formed a complex with the FlhI hexameric ring and facilitated the ring formation and the ATPase activity of FlhI. From the genetic analyses, we determined the FlhJ-FlhI interaction sites. We will discuss the structure of the FlhJ-FlhI complex, possible roles of FlhJ in protein export, and similarities between the flagellar protein export apparatus and F₀F₁-ATP synthase.

2P-030 藍色細菌 *T. elongatus* の時計蛋白質 KaiB における機能と動的構造との相関の解析

Analysis of the correlation between the function and structural dynamics on the clock protein KaiB from a cyanobacterium *T. elongatus*

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The circadian clock is an endogenous biological mechanism that generates autonomous daily cycles of physiological activities that reflect daily environmental cycles of light and temperature. It was recently reported that in a cyanobacterium its circadian clock with the 24-hour cycle is regulated only by the related component proteins, KaiA, KaiB and KaiC, in addition to the well-known negative feedback in their genes. The crystal structures of the three proteins were already known, but their static structures alone did not reveal details about the fundamental mechanism of the circadian rhythm. Alternatively, it has been suggested that the mechanism of the protein-clock can be understood by analyzing the dynamics in the molecules. We are assuming that some dynamics of KaiB regulates the circadian rhythm through the interaction with KaiA or KaiC in a dynamics-dependent manner from the results of various mutational analyses. To confirm this idea, I have been applying NMR, having a good advantage in analyzing dynamics of proteins at the atomic level, to analyzing the dynamics of KaiB. So far, I have assigned the main-chain resonances for ²H/¹³C/¹⁵N-labeled KaiB by NMR and revealed that the C-terminal regions of KaiB that might be involved in the interactions with KaiC are flexible. To elucidate their details, I introduced ¹³CH₃-labels into the deuterated KaiA or KaiB and analyzed the interactions of them with KaiC. Here, I report the results obtained from the analyses of KaiB.

2P-031 分子動力学/結合自由エネルギー計算によるヒト血清アルブミン-warfarin 結合に及ぼす脂肪酸の影響解析

Analysis of the effect of fatty acids on HSA-warfarin binding using molecular dynamics/binding free energy calculations

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Human serum albumin (HSA) is a major protein component of blood plasma, and binds with drugs and fatty acids (FAs). Drug binding to HSA can be modulated by cobinding of FAs; therefore it is important to clarify in detail the effect of bound FAs on the binding of a drug to HSA. In this study, we used molecular dynamics simulations and the binding free energy calculations to analyze the relationship between HSA-warfarin binding affinity and the bound FA molecules. Multiple HSA-warfarin complexes with different numbers/positions of bound myristates were prepared. The HSA-warfarin binding free energy was calculated for each complex and compared. Under the excess of the bound FA molecules, the magnitude of the binding free energy was smaller than that in HSA-warfarin complex without myristate. The unfavorable binding of warfarin to HSA was caused by the binding of myristate to the third highest affinity FA binding site, which was nearest from warfarin binding site. The decrease in the binding free energy was also explained from the atomic fluctuation at warfarin binding site. On the other hand, the magnitude of HSA-warfarin binding free energy was largest when two myristates were bound to the first and the second highest affinity FA binding sites. In this complex, electrostatic contribution as well as van der Waals contribution was favorable in the binding of warfarin to HSA. Thus, the present study provides insights into the effect of bound FAs on HSA-warfarin binding affinity.

2P-032 基準振動に基づく蛋白質-蛋白質相互作用の動的特徴の解析

Analysis of dynamic characteristics of protein-protein interactions

derived from normal modes

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Most of our understandings of protein-protein interactions in three dimensions were obtained from analyses of “static” protein-protein complex structures determined by X-ray crystallography. However, proteins are not static, and they fluctuate over a distribution of possible states. Therefore, the knowledge of dynamic characteristics of protein-protein complexes will be necessary for deeper understanding of protein-protein interactions.

To obtain the dynamical characteristics of protein-protein interfaces, in this study, we carried out normal mode analyses for 74 non-redundant hetero-dimers and for the artificial complex models generated by docking for these hetero-dimers. We examined a correlation of fluctuations between inter- or intra-molecular atoms existing in the interacting region to identify the correlation patterns of the fluctuations in the interfaces. We also compared the intra-correlations in the dimer state and those in the monomer state to examine the changes of the fluctuations in the formation of the interfaces. For the artificial complex models, the above investigations were also performed, and the results were compared to those of the native dimers. We found that there are several correlation patterns in the 74 hetero-dimers, and that the negative correlations of fluctuations in interacting regions may play an important role in the formation of the interfaces.

2P-033 タンパク質立体構造上でのリガンド構造アセンブルによるリガンド結合部位の予測

Ligand fragment assembly to predict ligand binding sites on protein three-dimensional structures.

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Structural information of proteins is rapidly growing due to recent structural genomics projects. However, a large number of proteins are still uncharacterized. Prediction of their ligand binding sites is the first step toward the identification of their functions. In this study, we have developed a method for predicting protein ligand binding sites and its binding mode by assembling the ligands based on the observed spatial distribution of “parts” on protein structures in known protein-ligand complexes in PDB. In this study, we considered three successive atoms as the parts of ligand. In this approach, we assume that ligand recognition follows the universal rules of molecular interactions regardless of protein families. We applied our method to 577 binding sites containing 112 kinds of ligands appeared in scPDB, an annotated database of druggable binding sites from PDB, and the performance was evaluated by the minimum distance and by the RMSD, between predicted and native ligand. When the minimum distance is less than 1Å or RMSD < 5.0Å, the prediction is considered to be successful in position or in conformation, respectively. As a result, 86% and 39% of the binding sites were successfully predicted in position and in conformation. These results imply that geometrical patterns of physicochemical interaction obtained from non-homologous complexes are quite effective for predicting protein ligand recognition.

2P-034 分子動力学法を用いた酸化型アズリンー還元型シトクロム c₅₅₁ 複合体の熱力学的安定性

Molecular dynamics study on thermodynamical stability of Oxidized Azurin – Reduced Cytochromec₅₅₁ complex

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Biomolecular binding process as shown in the formation of protein complex is one of the most important process for the expression of biomolecular function. For example, docking of Azurin(Az) and Cytochromec₅₅₁(Cyt) proteins occurs the electron transfer between the active sites of those proteins. For understanding of such formation process of protein complex, the observation of the structure and the free energy change of the Az-Cyt complex in solution should be needed. However, the actual complex structure and thermodynamical stability are not shown due to the difficulty of experimental observation. In this study, we carried out the molecular dynamics simulations for the oxidized-type Az(Az_{ox}), reduced-type Cyt(Cyt_{red}) and Az_{ox}-Cyt_{red} complex in water solution, and estimate the thermodynamical properties of these proteins in water solvent such as the internal energy, entropy, and solvation free energy of the proteins to reveal the thermal stability of the Az_{ox}-Cyt_{red} complex. We use the energy representation method for estimation of the solvation free energy of the proteins. The effect of the excluded volume and accessible surface area of the proteins are also shown. From these calculations, we estimate the thermal stability and the binding energy for the formation of the Az-Cyt complex.