systematic way. We have also compared the molecular properties with evolutionary information and essentiality of proteins within the context of network. We will present recent results of these analyses.

2P-064 *Streptococcus sobrinus* グルコシルトランスフェラーゼ-I のデキストラン結合に伴う熱容量変化

Heat capacity change for the binding of *Streptococcus sobrinus* glucosyltransferase-I to dextran

Hideyuki Komatsu (Dept Bioscience & Bioinformatics, Graduate School of Computer Science & Systems Engineering, Kyushu Institute of Technology)

Glucosyltransferase-I (GTF-I) secreted by mutants streptococcus catalyzes the transfer of glucosyl units from the cleavage of sucrose to a growing glucan chain.

Sucrose + (Glucose)_n → Fructose + (Glucose)_{n+1}

GTF-I (175 kDa) is composed of two functional domains: an N-terminal catalytic domain (GSd, 110 kDa, GS for glucansucrase) and a C-terminal glucan-binding domain (GBd, 65 kDa). The binding of the GBds to dextran ($\alpha(1-6)$ glucan) was previously studied by using isothermal titration calorimetry (ITC) to determine the changes of Gibbs energy, enthalpy, and entropy (ΔG , ΔH and ΔS) [1]. The result at 25 °C demonstrates that the strong binding ($\Delta G = -44$ kJ mol⁻¹) is driven by negatively large enthalpy ($\Delta H = -289$ kJ mol⁻¹) accompanied with large entropy loss ($-\Delta S = 245$ kJ mol⁻¹).

Herein, we reports the heat capacity change (ΔC_p) of binding, which parameter provides information about the changes in solvation and conformation of interacting molecules. ITC was used to determine the ΔH for the binding of GTF-I or its truncated proteins to dextran. Temperature-dependent ΔH was then measured, from which ΔC_p was calculated. The preliminarily estimated value was largely negative, suggesting release of large amount of hydrophobic hydration water. In this presentation, we will report more accurate ΔC_p , and discuss the changes in solvation and conformation of the protein and dextran upon their binding.

[1] Komatsu *et al.* *Biochemistry* (2007) **46**, 8436-8444.

2P-065 タンパク質内環境下における新しい pKa の第一原理計算による提案

Theoretical proposal of a new pKa concept applicable in protein environment by the first principles calculations.

Tomonori Tanaka (1), Katsumasa Kamiya (2), Yasuteru Shigeta (2) and Kenji Shiraishi (1). (1: Graduate School of Applied Physics, Univ of Tsukuba; 2: Institute for Picobiology, Graduate School of Life Science, Univ of Hyogo)

It is important to understand the behavior of protons in biomolecules for clarifying mechanisms of the physiological functions at the atomic level. However, accurate determination of the positions of protons and/or protonation states is still difficult for high-molecular-weight biomolecules such as membrane proteins. In such circumstances, pKa is commonly used to obtain information about protonation states of amino acid residues participating in physiological functions. Yet it should be noted that pKa is a thermodynamical parameter, defined originally for aqueous solution. The behavior of protons in protein environment is different entirely from that in aqueous solution in a sense that proton dynamics is much more constrained in the protein by its three-dimensional structure, suggesting that the use of the pKa definition in aqueous solution is inadequate for estimations of pKa in protein environment. Therefore, it is imperative to extend the pKa concept for the acquisition of the precise proton behavior under protein environment. In this study, we propose a new pKa concept, which is applicable in protein environment and compatible with the conventional pKa, on the basis of the first-principles calculations combined with the metadynamics approach that provide the precise estimations of free energies.

2P-066 リボヌクレアーゼ P タンパク質 Rpp21 と Rpp29 の複合体形成が触媒活性に重要である

The complex formation of Rpp21 and Rpp29 plays an important role in catalytic activity of ribonuclease P.

Takashi Honda (1), Yoshimitsu Kakuta (1,2) and Makoto Kimura (1,2). (1: Dept System Life Sciences, 2: Dept. Bioscience and Biotechnology, Graduate School, Kyushu University)

Ribonuclease P (RNase P) is a ribonucleoprotein complex involved in the processing of the 5'-leader sequence of precursor tRNA (pre-tRNA). The human RNase P protein subunits Rpp21 and Rpp29, which bind to each other, with a catalytic RNA (H1 RNA) are sufficient for activating endonucleolytic cleavage of pre-tRNA. Here we have determined the crystal structure of the complex between the *Pyrococcus horikoshii* RNase P proteins *PhoRpp21* and *PhoRpp29*, the archaeal homologs of Rpp21 and Rpp29, respectively. *PhoRpp21* and *PhoRpp29* form a heterodimeric structure, where the N-terminal two helices ($\alpha 1$ and $\alpha 2$) in *PhoRpp21* predominantly interact with the N-terminal extended structure, the β -strand ($\beta 2$), and the C-terminal helix ($\alpha 3$) in *PhoRpp29*. The interface is dominated by hydrogen bonds and several salt bridges, rather than hydrophobic interactions. The electrostatic potential on the surface of the heterodimer shows a positive charged cluster on one face, suggesting a possible RNA binding surface of the *PhoRpp21-PhoRpp29* complex. The present structure along with the mutational analysis suggests that the heterodimerization of *PhoRpp21* and *PhoRpp29* plays an important role in function of *P. horikoshii* RNase P.

2P-067 細菌べん毛フックキャップの結晶構造

Crystal Structure of Salmonella Flagellar Hook Cap, a Scaffolding Protein for

Hook Assembly

Hideyuki Matsunami(1,2), Katsumi Imada(1,3), Keiichi Namba(1,3). (1: Okinawa Institute of Science and Technology; 2: Dynamic NanoMachine, ICORP, JST; 3: Graduate School of Frontier Biosciences, Osaka Univ)

The bacterial flagellum is a large macromolecular complex composed of the filament, the hook and the basal body. Three capping proteins, FliD, FlgD and FlgJ, are required for assembly of the filament, the hook and the rod, respectively. FlgD is essential for polymerization of FlgE to form the hook; FlgE is excreted to the cell exterior without polymerization in the absence of FlgD, suggesting that FlgD forms the hook cap to facilitate the folding of exported FlgE in unfolded forms and its incorporation into the growing end of the hook. To understand the mechanism of the hook cap for hook assembly, structural information of FlgD is essential. Here we report a crystal structure of Salmonella FlgD in its pentameric form. Diffraction data were obtained at the SPring-8 beamline BL41XU and the structure was determined at 3.6-Å resolution. The electron density map revealed a "mushroom-shaped" structure of the FlgD pentamer. The N-terminal half of FlgD forms the stalk of the mushroom as a bundle of α -helices, although unambiguous model building is rather difficult due to relatively low resolution of the map of this portion. A flexible conformation of this region might play an important role in the hook capping mechanism. The C-terminal half, which is better resolved, forms the cap portion of the mushroom with a diameter of 90 Å, approximately a half of the hook. We will discuss possible mechanisms of the FlgD cap for efficient hook assembly as well as the similarities and differences between the three caps for flagellar assembly.

2P-068 Dynamics of amino acids in the electron transfer pathways between Oxygenase and Ferredoxin components in Rieske oxygenase system

Dynamics of amino acids in the electron transfer pathways between Oxygenase and Ferredoxin components in Rieske oxygenase system

Wayne Dawson (1), Tohru Terada (2) and Kentaro Shimizu (1). (1: Department of Biotechnology; 2: Agricultural Bioinformatics Research Unit, Graduate School of Agricultural and Life Sciences, The University of Tokyo)

Oxygenases (Ox) are involved in many metabolic pathways associated with the degradation of aromatic hydrocarbons; typically to detoxify them. Ferredoxin (Fd) is notable for its role in such processes as photosynthesis. Both contain a non-heme Fe₂S₂ that can be used to transfer electrons. Here we examine refinements of the X-ray structure of carbazole 1,9a-dioxygenase (CARDO) using MD simulation to understand the stability and dynamics of the amino acids; particularly those that are likely to permit electron transfer between the Ox and Fd subunits. The structure consists of three subunits of Fd:Ox forming. We find in this study that the general structure of CARDO remains stable during the simulation with a maximum rmsd of approximately 2 Å (all C α /C β) from the observed X-ray structure. In addition, the main residues that are thought to be located along the electron transfer path appear to interact with each other regularly during the 5 ns simulation permitting a proton conduction pathway.

2P-069 モルフォロジーを利用した蛋白質表面の様々な深さのポケットを同時に計算するアルゴリズムの開発

A fast algorithm for detection of pockets with multi-scale depth on protein surface by mathematical morphology

Takeshi Kawabata(1). (1. Grad.Sch.Info.Sci., NAIST)

Detection of pockets on protein surfaces is an important step to find putative binding sites of small molecules. Last year, we proposed a novel algorithm using small and large probe spheres (Kawabata and Go, *Proteins*, 68:516,2007); the pockets is defined as the region where the small sphere can enter, but a large sphere cannot. The radius of large probe is considered as the "depth" of pockets. However, a depth of binding pockets is widely distributed, and each type of binding molecule has a specific preference for the pocket depth; coenzymes like deep pockets, but peptides/oligosaccharides like shallow ones. If a type of binding molecule is unknown, we have to search for pockets with various depth values. In this study, we invented an efficient pocket detection algorithm for multi-scale probe pockets, which simultaneously calculate pockets with several different depths. The algorithm is based on the mathematical morphology for the 3D grids of proteins and probes, which have been applied for the protein pockets by Masuya and Doi (*J.Math.Graph.*13:331, 1995). The efficient multi-scale algorithm is constructed by the inclusion relationship of pockets: pockets for smaller spheres are parts of those for larger spheres. Using this algorithm, we can quickly calculate shallowness of pockets, Rinaccess (minimum radius of inaccessible probe spheres) for the protein residue and atoms, which provides clues to predict types of binding molecules.

2P-070 Effect of kosmotropic and chaotropic solutes on an allosteric protein under non-denaturing conditions

Effect of kosmotropic and chaotropic solutes on an allosteric protein under non-denaturing conditions

Antonio Tsuneshige (1) and Yuuki Yashiro (2): (1: Dept of Frontier Biosciences, Faculty of Biosciences and Applied Chemistry, Hosei University; 2: Materials Science, Faculty of Engineering, Hosei University)

We have studied the impact of several kosmotropic as well as chaotropic solutes under non-denaturing conditions on the allosteric function of human hemoglobin (HbA), namely, oxygen affinity for the first (K1) and last (K4) oxygenation steps,