Introduction of disulfide bonds into proteins is important for their folding and stability. Recently, it has been revealed that the mitochondrial intermembrane space (IMS) contains a disulfide-bond relay system consisting of Tim40/Mia40, Erv1, and Hot13, which allows disulfide bond formation in proteins imported into the IMS. Although Tim40 directly oxidizes newly imported substrates and Erv1 oxidizes Tim40, the precise mechanism of the action of the Tim40-Erv1 system still remains elusive. Here, we report the crystal structure of Tim40 truncated mutant, Tim40C4, N-terminally fused to the maltose binding protein (MBP) at 3 Å resolution. The structure of Tim40C4 is fruit-dish-like shape and composed of a long N-terminal loop and two anti parallel α -helices. The loop structure contains a putative active center, CPC motif, where a ring-shaped electron density is observed. The two helices following the loop are connected by two pairs of disulfide bond. Tim40C4 has a hydrophobic concave region formed by the hydrophobic side chains of Phe311, Phe315, Phe318, Val319, Phe334, Met337, and Phe341 protruding from the two helices. Interestingly, the linker segment between MBP and Tim40C4 is accommodated in this concave region, forming an α -helical structure. Therefore this hydrophobic region may well function as a substrate binding site, with which the linker segment associates as a pseudo substrate. Replacement of one of the four Phe in the hydrophobic region with Glu resulted in severe defects (F315E, F318E) or temperature sensitive defects (F311E, F334E) in cell growth. In vitro import experiments with mitochondria with Tim40 also suggest that the assembly of small Tim proteins into TIM22 complex is retarded by F311E mutation.

3P-031 分子動力学計算を用いた LILRB1/HLA-G と LILRB2/HLA-G の結合機構の違いに関する研究

Molecular dynamics simulation studies on the differences in the binding mechanism of LILRB1/HLA-G and LILRB2/HLA-G

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HLA-G, which is a member of non-classical human MHC class I molecules, is expressed on placenta, thymus and some tumors. It consists of three molecules; a heavy chain, β_2 -microglobulin (β_2m), and a short peptide derived from proteolytically degraded proteins inside a cell. HLA-G can suppress immune responses by binding to the inhibitory receptors, LILRB1 (LIR1) and LILRB2 (LIR2). The LIR binding sites of HLA-G are the α_3 domain of the heavy chain and β_2m . The LIR1/HLA-G recognition mechanism is different from LIR2/HLA-G. Here, we performed all-atom MD simulations of LIR1/HLA-G and LIR2/HLA-G complexes to investigate the differences. We used the crystal structure of LIR2/HLA-G, and a modeling structure of LIR1/HLA-G built by using the crystal structure of LIR1/HLA-A complex as a template. In the simulations, the interaction between the LIR1 and the α_3 domain is weaker than the interaction between the LIR2 and the α_3 domain, and the fluctuation of LIR1/HLA-G complex is larger than the LIR2/HLA-G one. Our results are consistent with experimental data. We will present the detail of the results at our poster.

3P-032 高分解能結晶構造によるフェリチンにおける鉄放出の分子機構の解明

Atomic Resolution Structures Indicate A Molecular Mechanism for Iron Release Through Functional Pore in Ferritin.

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Ferritins form large spherical protein nanocages composed of 24-subunits that concentrate iron in the internal cavity with 8 nm in diameter as ferric oxide mineral. When iron is needed in cells for the synthesis of the iron-containing co-factors, ferritin releases iron. Iron release, including mineral dissolution and iron exit from the internal cavity through the cage, is regulated by folding/unfolding of 3-fold pores formed from residues 110-134 in the C- and D-helices contributed by three subunits. The mechanism of iron release, however, is poorly understood compared with the iron uptake that is coupled with iron/oxygen oxidoreductase activity. Here, we report the structural and functional properties of pore mutants that provide insight into the mechanisms of pore gating and iron release from ferritin. Using the well characterized frog M ferritin model, we created three mutants of conserved pore residues, measured the rates of iron release and determined the high-resolution (1.3-2.0 Å) crystal structures and observed: 1) In the two pore mutants, Asp122Arg and Leu134Pro, increased disorder in the pore region in the crystal correlated with faster rates of iron release in solution, confirming that pore unfolding accelerates iron release. 2) Unexpectedly, Arg72Asp mutation, which also increased the rate of iron release to the same extent as observed in the other pore mutants, caused increased disorder of the N-terminus, with hydrogen bonding to Arg72. Thus, in addition to the Arg72-Asp122 salt bridge and the Leu134-Leu110 hydrophobic interactions that regulate pore gating in ferritin iron release, the results add a hydrogen-bond network that

couples the N-terminus to pore gating. Support for the work: NIH grants GM48958 (TA) and DK20251 (ECT) and JSPS (TT).

3P-033 オンサーガー・マハラップ作用とレプリカ交換を組み合わせたバースサンプリング：モデル計算

Transition path sampling using the Onsager-Machlup action with replica exchange: Model calculations

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To understand the functions of biomolecules (chemical reactions, conformational change, ligand-binding, protein-protein interactions etc.), defining a reaction path or reaction coordinate is an essential step. However, conventional molecular dynamics simulations often fail to find appropriate reaction paths even with huge computational resources. This is because such reactions occur much more slowly than computationally feasible time, and sampling efficiency of such reaction paths can be low especially for large systems. Hence it is desirable to seek alternative approaches for searching reaction paths. One such approach is the Onsager-Machlup (OM) action approach introduced by Ron Elber in this community of atomistic simulations. However, the minimization of the OM action is usually done and only a single (or few) path is obtained and analyzed even if the system is fluctuating at finite temperature. Another problem with the minimization is that the obtained path can be easily trapped around the initially guessed path, leading to unphysical results. According to the OM method, not only a single path but also a path ensemble can be obtained but such an investigation has been rarely carried out. Regarding the OM action as an effective Hamiltonian, we here propose to combine the OM method with a powerful sampling method, replica exchange. We illustrate the principle and effectiveness of our new method using some model systems and small peptide systems.

3P-034 脂肪酸 β 酸化酵素複合体構造変化の分子動力学シミュレーション

Molecular dynamics simulation of conformational change in a fatty acid β -oxidation multienzyme complex

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Recent biochemical studies suggest that many enzymes are organized into multienzyme complexes in the cytoplasm or subcellular organelles. Despite importances in cellular mechanisms, those structural bases to account for efficient enzymatic mechanisms have not been established yet. Among them, a fatty acid β -oxidation multienzyme complex (FOM) has been the subject of intense investigation, because of the importance of its function in the catabolic processes for fatty acids utilization, i.e., a HUB in the metabolic network. Defects of FOM lead to several well-known metabolic disorders including metabolic syndrome which has recently become popular. The purpose of this investigation is to clarify the mechanism of the conformational changes in FOM occurring in the process of the multi-enzymatic reactions at the atomic level by using molecular dynamics simulation. The structure of the $\alpha 2 \beta 2$ hetero complex of FOM, consisting of 2-enoyl-CoA hydratase (ECH), L-3-hydroxyacyl-CoA dehydrogenase (HACD), and 3-ketoacyl-CoA thiolase (KACT), was determined in several forms by Morikawa's group in 2004 and 2006. In this presentation, we show the simulation results of 50 ns molecular dynamics simulation for (i) FOM without ligands and (ii) FOM with 2 NAD⁺ in different forms (I & II), and discuss the conformational changes in FOM in the context of the multi-enzymatic mechanism.

3P-035 自由エネルギー変分原理に基づく結合自由エネルギー予測と化学的性質によるリガンドの分類

Prediction of binding free energy based on a free energy variational principle and classification of ligands by chemical property

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In order to estimate binding free energy between a target protein and a ligand in a drug discovery process, a technique free from adjustable parameters and computational costs is required when many molecules should be treated. So far, we have proposed a method based on a free energy variational principle for this objective. In this study, we examine what extent of the differences in ligand molecules can be regarded at small perturbations based on classification of ligands. The following formula express the free energy variational principle; $\langle H_1 - H_0 \rangle \leq G_1 - G_0 \leq \langle H_1 - H_0 \rangle_0$, where G_0 , H_0 , G_1 and H_1 denote free energy of a unperturbed system, Hamiltonian of the unperturbed system, free energy of a perturbed system and Hamiltonian of the perturbed system respectively. When the perturbation is small, i.e., the difference in physico-chemical property between ligands is small, the above formula can be approximated by $\langle H_1 - H_0 \rangle_1 \approx G_1 -$