and docked into the crystal structure of catalytic domain. Then, a spherical box of water molecules was placed around the subsites. Molecular dynamics simulations in explicit water were carried out at 300 K, and stable structures were chosen from the trajectories by using the cluster analysis. The key residues for the oligosaccharide recognition based on the subsite theory were identified. In the bound oligosaccharide, the glucose unit on the non-reducing end was more fluctuated than that on the reducing end. Taken together with the experimental results of binding thermodynamics, the energetic contribution on the respective subsites could be elucidated.

1PT131 α ヘリックス・ β ストランドレプリカ交換分子動力学シミュレーション法の開発と応用

Development and application of the helix-strand replica-exchange molecular dynamics method

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Biomolecules such as proteins have complicated free energy surfaces with many local minima. Conventional molecular dynamics (MD) and Monte Carlo (MC) simulations tend to get trapped in these local-minimum states. One of the powerful techniques to avoid this difficulty is the replica-exchange method. We propose helix-strand replica-exchange molecular dynamics method. We apply this method to a design peptide and show the results.

1PT132 PDB データベースによるアミノ酸ごとに最適化した主鎖二面角力場 Development of a force field of backbone dihedral angles for each amino acid using Protein Data Bank

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A force field is widely used in the field of molecular simulations for biomolecular systems and is determined by an atomic model based on classical mechanics. Many commonly used force fields for protein systems such as AMBER, CHARMM, GROMACS, OPLS, and ECEPP have amino-acid-independent force-field parameters of main-chain torsion-energy terms. Here, we propose a new type of amino-acid-dependent torsion-energy terms in the force fields. As an example, we applied this approach to AMBER ff03 force field and determined new amino-acid-dependent parameters for ϕ and ϕ' angles for each amino acid by using our optimization method, which is based on the minimization of some score functions by something in the force-field parameter space, where the score functions are derived for the protein coordinate data in the Protein Data Bank (PDB) [1-3]. In order to test the validity of the new force-field parameters, we then performed folding simulations of α -helical and β -hairpin peptides, using the optimized force field. The results showed that the new force-field parameters gave structures more consistent with the experimental implications than the original AMBER ff03 force field.

[1] Y. Sakae and Y. Okamoto, Chem. Phys. Lett. 382, 626-636 (2003).

[2] Y. Sakae and Y. Okamoto, J. Theo. Comput. Chem. 3, 339 (2004).

[3] Y. Sakae and Y. Okamoto, J. Theo. Comput. Chem. 3, 359 (2004).

1PT133 コフィラクチンフィラメントの分子動力学モデルの構築 Molecular dynamics model of cofilactin filament

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Cofilin is an actin regulatory protein which disassembles and severs actin filaments. The binding affinity of cofilin to actin filament is regulated via biochemical pathways. In addition, it has been pointed out that tension in actin filament prevents cofilin from binding to the filament. Because cofilin binding put a twist on the helix of the actin filament that might be incompatible filament conformation caused by the tension, we suppose that structural change of actin filament is related to the mechanism on tension-induced inhibition of cofilin binding. In order to obtain better understanding the mechanism at molecular level, we need to investigate energetic and structural effects on binding domains of actin and cofilin caused by the conformational change of the filament.

To achieve the research mentioned above, in this study, we construct molecular dynamics (MD) model of cofilin-decorated actin (cofilactin) filament. First, we placed cofilins in the vicinity of their binding domains on actin along the filament based on the PDB data of actin-cofilin complex. Second, MD simulation for 20ns was conducted to obtain an equilibrium structure of cofilactin filament. Because this equilibrium structure has included thermally-

induced various twist angles, a cofilin-bound actin trimer with a moderate angle has been extracted from the equilibrium structure as a sample of cofilactin complex. In this way, we succeeded to construct MD models of cofilactin complexes with distinct twist angles.

1PT134 ロボット機構の運動学に基づくタンパク質の内部運動解析 Internal Motion Analysis of Proteins based on Robot Kinematics

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From the perspective of robot kinematics, we investigated a method for predicting the internal motions of proteins using data from PDB. The protein model used in this method is simple; it comprises main chains and linear springs between the alpha carbons. The main chains are considered to be robot manipulators that control the positions of the alpha carbons or the distances between them, where the dihedral angles on the main chains correspond to the joint angles of robot manipulators. We apply forces in static equilibrium to the model and evaluate the deformation caused by them. The deformation magnitude differs depending on the direction of the force, despite the magnitude of applied forces being the same. It can be concluded that deformations of a large magnitude occur easily. Here, forces are applied in two different manners. One is the application of forces at the alpha carbons in the residues interacting with other molecules and the other is the application of forces at the centroids of the atomic groups (e.g., secondary structures, domains, and subunits). The formulation of basic equations for this method is similar to that for kinematic analysis of robot manipulators. We applied this method to data from PDB of some proteins (e.g., calmodulin, lactoferrin, and aspartate transcarbamoylase). Despite being a simple model, the results are almost consistent with the actual internal motions.

1PT135 コンピュータシミュレーションによる 4-helix bundle タンパク質を基にしたタンパク質分子間相互作用面の設計

Simulation study for designing protein-protein interaction interfaces based on a four-helix bundle protein

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Some proteins have the ability to self-assemble and form fibers. The purposes of our study are: i) designing binding interfaces on two proteins which specifically interact to each other; ii) inducing self-assembly through the intermolecular interaction between the two proteins; and iii) forming co-polymers. A four-helix bundle structure is chosen as a model for designing the binding interface. Using the four-helix bundle as the scaffold, we designed the binding interfaces on the target protein YciF involving charged and hydrophobic amino acid residues. To evaluate the designed binding interfaces, we performed molecular dynamics (MD) simulation of the variants of YciF proteins with the designed binding interface using GROMACS [1]. For the initial state, two protein molecules with the designed interface were placed with appropriate distance, and their binding interfaces faced each other. After 5ns simulation, we calculated the distance between centroid of binding interface of the molecules, and visualized the structures using Pymol [2]. The details will be presented at the conference.

[1] Berendsen, et al. Comput. Phys. Commun. 91 (1995) 43. [2] <http://www.pymol.org>

1PT136 透明で柔軟性を持った実用性の高いタンパク質分子模型 A Soft and Transparent Handleable Protein Model

Masaru Kawakami (Sch. Mat. Sci.)

The field of molecular and structural biology currently relies on computer-generated graphical representations of three-dimensional (3D) structures to conceptualize biomolecules. As the size and complexity of the molecular structure increases, model generation and peer discussions become more difficult. It is even more problematic when discussing protein-protein interactions wherein large surface area contact is considered. This report demonstrates the viability of a new handleable protein molecular model with a soft and transparent silicone body similar to the molecule's surface. A full-color printed main chain structure embedded in the silicone body enables users to simultaneously feel the molecular surface, view through the main chain structure, and manually simulate molecular docking. The interactive, hands-on

experience deepens the user's understanding of the complicated 3D protein structure and elucidates ligand binding and protein-protein interactions. This model would be an effective discussion tool for the classroom or laboratory that stimulates inspired learning in this study field.

1PT137 蛋白質熱安定性に及ぼす糖の影響

Effects of sugars on the thermal stability of proteins

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It is experimentally known that the heat-denaturation temperature of a protein T_m is raised by the sugar addition. The magnitude of this effect stabilizing the protein depends on the sugar species [1]. In earlier work, we proposed a measure of the thermal stability of a protein, which is defined as the solvent-entropy gain at 298 K upon protein folding S normalized by the number of residues [2,3]. S was calculated using a hybrid of the angle-dependent integral equation theory combined with the multipolar water model and the morphometric approach. Here we show that S can be calculated using the hard-sphere solvent whose number density and molecular diameter are set at those of water. We then investigate the effects of sugar addition on the thermal stability by considering water-sugar solution modeled as a binary mixture of hard spheres. The three-dimensional integral equation theory is employed. The thermal stability is determined by a complex interplay of the molecular size of the sugar D and the total packing fraction of the solution η . D is estimated from the volume per molecule in the sugar crystal, and η is calculated using the experimental data of the solution density. We find that the thermal stability is considerably enhanced by addition of sucrose. In the presentation, we will discuss the effects of addition of not only sugars other than sucrose but also osmolytes.

[1] J. F. Back, *Biochemistry* 18 (1979) 5191.

[2] K. Amano *et al.*, *Chem. Phys. Lett.* 474 (2009) 190.

[3] K. Oda *et al.*, *J. Chem. Phys.* 134 (2011) 025101.

1PT138 ヒトカルシトニンアミロイド線維形成阻害機構の解析

Amyloid fibril inhibition mechanism of human calcitonin

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Human calcitonin (hCT) is a 32-residue peptide hormone. hCT is used as a medicine for osteoporosis, but it easily forms amyloid fibrils under physiological condition, which is a problem on medication. Salmon calcitonin (sCT) is known to inhibit amyloid formation of hCT. Combinational use of hCT and sCT is expected to improve the pharmacological activity. While the N-terminal region (9-19) of sCT takes an α -helix, the C-terminal half of the helix is disordered in hCT. Although an interaction between the disordered region and the C-terminal tail of hCT is proposed to play a role on the amyloid formation, the detailed mechanism of the inhibition of the amyloid formation by sCT has not been revealed. In this study, we investigated amyloid fibril formation of hCT in the presence of each of two chimera peptides, which are comprised of the 1-15 residues of hCT and the 16-32 residues of sCT and, the reverse combination. The former region (1-15) contains the stable α -helix and the latter the flexible region in hCT. Amyloid fibril formation was monitored by fluorescence intensity from Thioflavin T. While the fluorescence intensity raised about 30~40 hours after incubation of hCT alone, the amyloid fibril formation was inhibited in the presence of sCT, or each of the two chimera peptides, indicating that each of the N-terminal and the C-terminal regions of sCT possesses the inhibitory ability. From these results, we conclude that both the C-terminal region and the N-terminal stable α -helix are engaged in the inhibition of the amyloid formation.

1PT139 ダブル pH ジャンプによるアミロイド線維のほぼ可逆的な構造変化の解析

Analysis of Almost Reversible Conformational Change of Amyloid Fibrils by Double pH-jump

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To obtain insights into the mechanism of amyloid fibril conversion, pH-jump experiments were performed using a H2 peptide of mouse prion protein. Previously, we reported that H2 peptide formed ordered amyloid fibrils with an

immensely large minimum at 207 nm on CD spectrum at pH 2.9 (named pH2.9 fibrils), but aggregate-like fibrils with a minimum at 220 nm were formed at pH 7.5 (named pH7.5 fibrils) near its isoelectric point. In this study, firstly single pH-jump from 2.9 to 7.5 was performed. As a result, the CD spectrum showed that the pH2.9 fibrils instantly changed to pH7.5-like fibrils with a minimum at 218 nm, but the ellipticities were certainly distinct between pH7.5-like and pH7.5 fibrils, implying that the conformation of pH2.9 fibrils partially remains even at pH 7.5. Moreover, the pH7.5-like fibrils almost returned to the pH2.9 fibrils by restoring the solution pH from 7.5 to 2.9. FT-IR spectra indicated that these conformational changes were caused by the disruption of highly ordered β -sheet and β -turn conformations, and the subsequent their reconstructions. In addition, kinetic conformational changes of the fibrils after the single and double pH-jumps were examined using ANS fluorescence stopped-flow. As a result, these conversions of the fibrils composed of a few phases were accomplished within several seconds. Thus, amyloid fibrils can be changed readily between the distinct conformations separated by a low energy barrier almost reversibly.

1PT140 金属結合によるヒトプリオンタンパク質のペプチド断片の構造変化 Conformation change in peptide fragments of human prion protein caused by metal binding

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Prion diseases are transmissible spongiform encephalopathies (TSE) and lethal infectious diseases caused by misfolding of prion protein (PrP). Human prion protein (huPrP) binds metal ion at the residues 60-91 (His61, His69, His77, His85) and residues 90-126 (His96, His111) in N-terminal. Histidine residue are known as high-affinity sites to metal binding. Metal binding to PrP is considered as an important process for misfolding of PrP. This report aims to investigate conformation change in huPrP caused by metal binding. huPrP Peptide fragments containing His96 and/or His111 were examined for binding metal such as Cu^{2+} , Ni^{2+} and Co^{2+} . pKa values were determined with electro titrimetric analysis (Metrohm, 848 titrimo plus). Metal binding to peptide fragment was observed using visible absorption spectra (Hitachi, U-2800 Spectrophotometer) and Circular Dichroism (CD)(JASCO, J-820). Secondary structure of peptide fragment was observed using Far-UV CD. Addition of Cu^{2+} and Ni^{2+} to huPrP (93-102:GGTHSQWN) showed the shift of pKa value. Vis absorption and Vis CD indicated the characteristic spectra resulting from metal binding. It was derived from imidazole N bound to metal ion and from d-d transition of metal ion. Far-UV CD spectrum showed the conformational change of secondary structure induced by metal binding. We will also investigate metal binding to peptide fragments of huPrP containing His111 by using UV/Vis spectroscopy, CD and electron spin resonance (ESR) to further understand about metal binding to huPrP.

1PT141 ユビキチンの温度-圧力-自由エネルギー地形図

Temperature-pressure-free energy landscape of ubiquitin

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To understand the change of packing and/or solvation of protein denaturation from quantitative point, volume change ΔV and isothermal compressibility change $\Delta\beta_T$, and thermal expansion coefficient change $\Delta\alpha$ are needed. These thermodynamic parameters at given temperature and pressure are determined from temperature(T)- pressure(p)- free energy change(ΔG) landscape, which is obtained from combined series of pressure/temperature variable denaturation experiments.

In this study, we explore the ($T, p, \Delta G$) landscape of ubiquitin. FTIR spectra of ubiquitin was reversibly changed by temperature/pressure perturbation at pD 2.0 and pD 5.5. To determined ΔG at given temperature and pressure, temperature/pressure transition curves were obtained from change in FTIR peak intensity at 1673 cm^{-1} which is assigned to native β -strand structure. Three-dimensional ($T, p, \Delta G$) landscapes and thermodynamic parameters (ΔV , $\Delta\beta_T$, $\Delta\alpha$, entropy change ΔS and heat capacity change ΔC_p) at ambient temperature and pressure (298K, 0.1MPa) were obtained.

1PT142 X線小角散乱によるフェリチンアセンブリ反応の追跡

Ferritin assembly kinetics followed by small-angle X-ray scattering