of PH cyt *c* exhibited a periodical fluctuation. Thus, the fluorescence time traces from single PH cyt *c* molecules can be classified into several patterns of fluctuations, and demonstrate the variety of structural dynamics of PH cyt *c* in the time domain from microsecond to millisecond. We will also present of the results of the recent analysis on the single molecule dynamics of cytochrome *c* from *Pseudomonas aeruginosa* and bovine β -lactoglobulin.

3P063 The investigation of the relationship between non-local interaction and the effect of single alanine insertion in staphylococcal nuclease

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Structural elements extracted from SNase by the systematic alanine insertion analysis are expected to be fundamental units for structural formation. We assume that these regions are involved in the non-local interactions responsible for the tertiary structure formation. Non-local interaction is the consequence of several amino acid residues forming a cluster. In this study, in order to understand the origin of the non-local interaction encoded into the structural element, multiple substitution mutation analysis was performed. The region from 62 to 67 is a structural element on the α 1-helix. We prepared three single substitution mutants (T62A, M65A, and V66A), every possible double and triple mutant on these residues. According to the crystal structure, these residues are densely packed to each other. The far-UV CD spectra revealed that, while single and double substitution mutants folded into native structure, the triple substitution mutant was unstructured under physiological condition. The CD spectrum of the triple mutant was almost identical to those of the single alanine insertion mutants in this region, indicating that the effect of triple substitution on the tertiary structure can be assumed to represent those of single alanine insertion mutation. From these result, we proposed that the structural element is the determinant region of the native contact, where the alanine insertion in structural element highly influences the native residue-residue contacts to result in the loss of the foldability.

3P064 高時間分解能一分子測定による緑膿菌由来シトクロム *c* の変性状態内の運動観察

Denatured state dynamics of cytochrome *c551* observed by single-molecule measurement with improved time resolution

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Single-molecule fluorescence measurements can reveal the behavior of proteins that cannot be assessed by bulk measurements. To investigate the dynamics of unfolded proteins in the wide time domain, the short time resolution and the long observation time are both required. In this study, we first reduced the time resolution of the single molecule measurements from 30 ms to 2 ms by introducing a high-speed CCD camera. We next lengthened the observation time of single molecules from 1 s to 2 s by synchronizing the laser excitation and the trapping of the molecules in the observation area to minimize the bleaching of the dye. We investigated the conformational dynamics of cytochrome *c551* from *Pseudomonas aeruginosa* (Cyt *c551*) in the unfolded state, which was labeled by a fluorophore, ATTO, whose fluorescence is quenched by heme depending on the conformation. We first measured single molecule fluorescence from ATTO by using the developed system, and conducted the same experiment for the labeled Cyt *c551*. The comparison of the data suggested that the conformational fluctuation of a protein contributes to the fluorescence intensity changes. Further experiments are underway to reveal the properties of the conformational dynamics of Cyt *c551*.

3P065 Folding of equine β -lactoglobulin entrapped in wet silica gels studied by circular dichroism spectroscopy

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Wet silica gel is porous and transparent to far-UV so that the entrapped proteins are hydrated and can be monitored by spectroscopic measurement. Previously, Shibayama has shown that within wet silica gels the refolding rate of bovine β -lactoglobulin (BLG) was significantly slowed down (1). In this study we observed the refolding reaction of equine β -lactoglobulin (ELG) entrapped in wet silica gel monitored by circular dichroism (CD). β -lactoglobulin predominantly consists of β -sheet in its native state. ELG forms the molten globule (MG) state with excess nonnative α -helices under acid denaturing condition, and it shows equivalent properties of the kinetic folding intermediate which is formed within the dead time of the stopped-flow CD measurements (2). In the gel, the refolding rate of ELG was slowed down and the formation

of nonnative α -helices was detected. The CD spectrum of the first accumulated intermediate was similar to that of MG state in silica gel as in solution. However, these two CD spectra in the gel were different from corresponding spectra in solution. Furthermore, the CD spectrum under the native condition was also different from the corresponding spectrum in solution. These results suggest that molecular properties of ELG are similar between the silica gel and solution but the silica gel environment influences not only the folding rate but also structures of folding states.

Reference

- (1) Shibayama (2008) FEBS Lett. 582, 2668-2672
- (2) Fujiwara et al. (1999) Biochemistry 38, 4455-4463

3P066 Unfolding of β -hairpin peptides by pressure: FT-IR and FRET studies

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Applying hydrostatic pressure to proteins has long been known to result in full or partial destabilization of the native structure. However, the mechanism is still unclear. Complexity of protein structures confounds understanding of the mechanism. A study on model peptides forming secondary structures is a crucial step to elucidate the pressure effect on the protein structures. We previously revealed that pressure induces folding of α -helices of model peptides (Imamura *et al*, Proteins 75, 2009, 911-918 and Imamura *et al*, Biochim. Biophys. Acta. 1804, 2010, 193-198). The present study focuses on the β -sheet structure, which is one of the most fundamental secondary structures. By using pressure-variable infrared spectroscopy, we investigated β -hairpin peptides, chignolin (GYDPETGTWG) and GB1m3 (KKWTYNPATGKFTVQE), which have been studied as typical models of the β -sheet structure. Applying pressure to the peptides results in decreases in the amide I intensities at ~ 1680 cm^{-1} stemming from the antiparallel β -sheets. This indicates that the β -sheet is disrupted by pressure. We also monitored fluorescence resonance energy transfer (FRET) of a variant of GB1m3 termed GB1m3p, in the sequence of which the *p*-cyanophenylalanine residue at the position 12 and the tryptophan residue at the position 3 are a donor and an acceptor, respectively. The FRET efficiency of the peptide is 90 % at 0.1 MPa and decreases with increasing pressure. This indicates that pressure increases the end to end distance.

3P067 マルチカノニカルレプリカ交換法による小蛋白質の折れ畳みシミュレーション Folding simulations of a small helical protein by multicanonical replica-exchange method

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We are studying folding mechanism of a small helical protein HP36, which is the C-terminal half of chicken villin headpiece domain. We performed folding simulations of HP36 by use of multicanonical replica-exchange method (MUCAREM). We used CHARMM22 force field with CMAP backbone-torsion corrections for protein molecule, and 3513 TIP3P water molecules were included in the simulated system. Our simulations were started from the fully extended conformation of the peptide. During the production run for about 2 μ s, folding events into native-like conformations were observed several times. The least backbone RMSD from the X-ray structure was 1.1 Å.

We analyzed the simulation data, focusing on the inter-residue native contact formation, secondary structure formation, and the dehydration of some parts in the protein molecule. The results of the analyses suggested that (i) The formation of the C-terminal helix and the hydrophobic collapse take place at the earliest stage of folding, (ii) the backbone and the side chains near the loop between the central helix and the C-terminal helix take native-like conformations, and (iii) the side-chain packing at the hydrophobic core and the dehydration of the core side chains take place simultaneously at the later stage of folding.

3P068 イヌミルクリゾチームのアンフォールディング経路についての理論的研究：ヤギ α ラクトアルブミンとの比較 A theoretical study of unfolding pathway of Canine milk lysozyme: Comparison with the unfolding pathway of Goat α -lactalbumin

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Canine milk lysozyme (CML) and Goat α -lactalbumin (GLA) belong to C-type lysozyme/ α -lactalbumin family. In the native state, the backbone conformation of CML is similar to that of GLA, which has 41% sequence identity with CML. These proteins adopt the molten globule (MG) state as an interme-

diate of the unfolding process. A recent experimental study on the MG state of these proteins has shown, however, that the tertiary contacts of CML are different from those of GLA. In this study, we address the issue of the difference in the unfolding pathways between the homologous proteins. We investigate the unfolding process of CML at several temperatures using all-atom molecular dynamics simulations. We find that α -domain is more stable than β -domain in the native state. We also find that the unfolding process starts at β -domain. It corresponds to the higher stability of s-domain in the MG state shown in the experimental study. We discuss the unfolding pathways of CML and GLA in detail.

3P069 タンパク質製剤安定性分析に向けた体系的スクリーニング法 A systematic screening approach for analyzing protein formulation stability

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Aggregation of protein is a major problem in their use as drugs in the biopharmaceutical industry. Protein aggregation occurs readily in almost all biopharmaceutical processes. Aggregation levels as low as 1% over a 2 year shelf-life can render a product clinically unacceptable. This paper proposes a simply systematic approach for analyzing aggregation factors in protein formulations. The approach includes designing the optimal formulating conditions using DoE technique, determining the protein stability in stress and accelerated testings using FTIR and SEC, and analyzing the main effects of single factors and interaction effects of combined factors on aggregation. Our results indicated that (i) analysis at a low protein concentration is not always applicable to high concentration formulations; (ii) an investigation of interaction effects of combined factors as well as main effects of single factors is effective for improving conformational stability of proteins; (iii) with the exception of pH, the results of stress testing with regard to aggregation factors would be available for optimum formulation instead of performing time-consuming accelerated testing. (iv) an optimal pH condition should not be determined in stress testing but in accelerated testing, because of inconsistent effects of pH on conformational and storage stabilities. In summary, we propose a three-step strategy, using FTIR, SEC and DoE techniques, to effectively analyze the aggregation factors of proteins and rapidly screen suitable conditions of protein formulation.

3P070 Multi-scale free energy calculation method with the combination of coarse-grained and all-atom models.

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Free energy landscape of bimolecular systems characterizes conformational changes of the relevant biological functions. It is therefore important to investigate the landscape. Because of the complex interactions in the systems, the landscape is very rugged, i.e., there exist many local energy minimum states. When the landscape is investigated by the conventional molecular dynamics (MD) simulation, protein conformation tends to be trapped within a limited number of local energy minimum states.

To tackle this problem, we developed a new method to calculate the free energy landscape by using both coarse-grained (CG-) and all-atom (AA-) models. In this method, firstly, CG-MD simulation searches the rough landscape efficiently without the trappings. Then non-interactive independent AA-MD simulations investigate the details of the local landscape with massively parallel computations. Finally, each detail landscapes are combined so as the free energy landscape to be self-consistent.

We applied this method to chignolin and deca-alanine as benchmark tests and checked the efficiency of the free energy calculation. For applications to larger systems, we are calculating the folding landscape of villin headpiece subdomain HP35.

3P071 ポリアラニンのマルチバーリック・マルチサーマル分子動力学シミュレーション multibaric-multithermal molecular dynamics simulations of a polyalanine

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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 multicanonical algorithm. However, because the multicanonical simulation is performed in a fixed volume, neither the pressure dependence nor temperature dependence at certain pressure can be investigated. In order to overcome this difficulty,

I have recently proposed multibaric-multithermal (MUBATH) MC [1-3] and MD [4-7] algorithms. I applied the MUBATH MD algorithm to apolyalanine dipeptide in explicit water. I will discuss conformational changes of polyalanine induced by temperature and pressure changes. [1] H. Okumura and Y. Okamoto: Chem. Phys. Lett. 383 (2004) 391. [2] H. Okumura and Y. Okamoto: Phys. Rev. E 70 (2004) 026702. [3] H. Okumura and Y. Okamoto: J. Phys. Soc. Jpn. 73 (2004) 3304. [4] H. Okumura and Y. Okamoto: Chem. Phys. Lett. 391 (2004) 248. [5] H. Okumura and Y. Okamoto: J. Comput. Chem. 27 (2006) 379. [6] H. Okumura and Y. Okamoto: Bull. Chem. Soc. Jpn. 80 (2007) 1114. [7] H. Okumura and Y. Okamoto: J. Phys. Chem. B 112 (2008) 12038.

3P072 二面角空間における蛋白質分子の補償的ダイナミクス Compensating Dynamics of a Protein Molecule Observed in Dihedral Angle Space

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The motions of proteins are mainly caused by shifts of main-chain dihedral angles, φ , ψ , and ω . In a thermally fluctuating protein, the motion induced by each dihedral angle is always compensated for by motion produced by many other dihedral angles in order to maintain a stable globular structure. To describe the structural fluctuation of proteins in equilibrium state, principal component analysis (PCA) is widely used, which is performed by solving an eigenvalue problem of the Cartesian coordinate variance-covariance. Although the similar analysis is expected in dihedral angle space, PCA in dihedral angle space is very difficult because of existence of higher-order correlations among the dihedral angles. Thus, the mechanism of compensating dynamics in dihedral angle space had been unrevealed. In this study, to describe structural fluctuations by only two-body correlation among dihedral angles, we developed the dihedral variance-covariance matrix linearly transformed from the Cartesian variance-covariance matrix. The transformed matrix expresses the compensating dynamics and satisfies the condition for maintenance of the globular structure. From the analysis of Haemophilus influenzae ferric-binding protein (PDB ID: 1D9V, 1MRP) and decaalanine, it was found that such dihedral cooperation was essential feature for globular proteins but not for short peptides. By linear response theory using the transformed matrix as a response function, the prediction of the protein structural change upon ligand binding was improved.

3P073 Extracting thermodynamics information from multi-scale state space network

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In the last few decades, there have been significant interests in exploring the thermodynamics properties of different biological systems using simplified toy models that mimic their basic properties. Although, these studies have fundamental importance, most systems are complicated and toy model representation may fail to address the detail thermodynamic features of these complex systems. We believe that the establishment of a minimal physical model which takes into account the essential complexity in their kinetics or dynamics remains to be a challenging problem. In this work we present a new way to extract the nonequilibrium thermodynamic information directly from the experimental result without postulating a priori model. Our approach is based on the use of computational mechanics that enables us to construct the multiscale state space network (SSN) directly from time series. The constructed SSN has the important properties that it represents the minimal model which captures the dynamical essence in the time series. The thermodynamical properties of the multiscale SSN and its biological implications will be explored in this presentation.

3P074 一分子測定法による β -ラクトグロブリンの蛍光強度変化と拡散の観測 Single-molecule observation of the translation diffusion and fluorescence intensity of β -LG

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To understand the dynamics of protein folding, the information on the various conformational freedoms of proteins is required. The translational diffusion coefficient is one of such information and reflects the hydrodynamic radius of proteins. Thus, to increase structural information on the folding of proteins, we observed the fluorescence intensity change and the translational diffusion at the same time for bovine β -lactoglobulin (β -LG) based on single molecule