distribution of atomic contacts in native structure (e.g. Nakamura, Makabe et al. J Mol Biol 2010, Itoh and Sasai PNAS 2008). In ribosomal protein S6 of *Thermus thermophilus* (S6T) and its circular permutant, for example, the folding nucleus consists of a two-strand-helix motif which is recruited from different parts of the protein depending on the topology of the native structure. In S6 from *Aquifex aeolicus* (S6A), which has the topology similar to and atomic contacts different from S6T, on the other hand, the folding nucleus is similar to S6T permutants suggesting the importance of atomic contacts (Lindberg and Oliveberg, Curr Opin Str Biol 2007). We analyze such flexibility and diversity in folding mechanism by using the Wako-Saito-Munoz-Eaton model of folding. Free energy surface of folding is calculated with the two-dimensional representation (Itoh and Sasai, J Chem Phys 2009) by adopting two order parameters, one describing the degree of folding at a two-strand-helix motif and the other at the rest part of protein, and discuss how the folding route is modified upon circular permutations and changes in atomic contacts.

2P063 幾何学的構造を考慮した蛋白質フォールディングの解析的モデル
Analytical Model for Protein Folding improved by introducing topological factors

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The current understanding of the physical mechanisms controlling protein folding is strongly based on the energy landscape theory and the funnel concept. This theoretical framework provides the basis for understanding an entire new family of experiments that have been designed to provide a quantitative understanding of these mechanisms. In contrast to these studies, purely analytical studies add an important complement to our understanding of the interplay between geometrical and energetic effects in the folding landscape. A free-energy functional model based on polymer theories was developed by Plotkin and Onuchic. However, in this model, local geometrical information, e.g. existence of A-helix, cannot be estimated appropriately due to the mean field approximation for the configurational entropy. As the approximation is introduced by hand, it is difficult to improve our theory systematically. In this study, we have constructed an analytical scheme based on a Hamiltonian without an artificial term. Our model is based on a Gaussian filament with Go-like potential. This is possible to estimate configurational entropy systematically. The dependence of thermodynamical variables upon the contact map obtained by simulations is reproduced by our analytical model. We establish the relation between entropy and geometrical information of folded state.

2P064 網羅的アラニン挿入変異解析法によるジヒドロ葉酸還元酵素の機能エレメントの分類
Classification of the functional element of dihydrofolate reductase(DHFR) by the systematic alanine insertion

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To design an artificial protein, it is essential to elucidate the building blocks indispensable for function expression and structural formation, called functional and structural elements. We applied the systematic alanine insertion analysis to DHFR to extract these elements. The functional and structural elements have been qualitatively assigned by the TMP resistance assay and the solubility assay. In order to examine the properties of the functional and structural elements of DHFR more precisely, we examined the solution structures of the insertion mutants by CD and their enzymatic activities spectroscopically. Previously, the alanine insertion mutants of DHFR into the region between the 71st and the 86th residues which is defined as a structural element were purified. The several mutants exhibit the decrease of the enzymatic activity to 10%, despite that the TMP assay did not show any serious effect. It can be assumed that the functionality is composed of the following three categories: 1) DHF binding ability, 2) NADPH binding ability, and 3) Catalytic activity. In order to understand functional information involved in the elements precisely, the capability of DHF or NADPH binding to DHFR was examined by fluorescence titration. The K_d values for DHF binding of the mutants from 77 to 81 were significantly increased up to 31-fold to 61-fold of that of the WT. In addition, NADPH binding was also increased up to 91-fold to 262-fold. These results suggest that this functional element is responsible for the binding of both DHF and NADPH.

2P065 Glycerol-induced folding in four species of 1SS-variants of lysozyme - the potential folded structure remaining in unstructured proteins.

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Disulfide-deficient variants of lysozyme have been systematically studied by us. In general, two-disulfide (2SS) and one-disulfide (1SS) variants were substantially unstructured except the variant 2SS(1+2) containing two disulfide bridges, Cys6-Cys127 and Cys30-Cys115. However, ordered structures were induced in some parts of molecule by the addition of glycerol. This solvent additive is useful to study the potential folded structure remaining in unstructured proteins. It is likely that a part of molecule preferentially has a propensity to form an ordered structure, but it is not so significant to be detected by spectroscopic observations. The addition of glycerol may enhance the inherent propensity and enable us to observe the potential structure. CD spectra showed the glycerol-induced folding of unstructured 1SS-variants of lysozyme. In order to see the location of induced structure, we carried out H/D exchange reaction of 1SS-variants in a solution of 30% to 70 % glycerol, and then measured HSQC spectra using a mixture of 95% DMSO and 5% D₂O as a quenching buffer for H/D exchange. In cases of 1SS[64-80] and 1SS[76-94], protection factors for H/D exchange were about 10 to 20 at most residues even in a solution of 70% glycerol. In the case of 1SS[6-127], they increased to about 100 to 200 in the presence of 50% glycerol. The glycerol concentration-dependence of protection factors was measured for 1SS[6-127], and the glycerol-induced folding of 1SS[30-115] have been studied by monitoring the CD spectra and the H/D exchange rates.

2P066 巨大分子量タンパク質の構造安定性
1E1450 Conformational stability of large proteins

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Knowledge about conformational stability of proteins is accumulated from studies on small proteins less than 300 amino acid residues. There are good correlations between denaturation m value / denaturant heat capacity, ΔC_p, and molecular size / changes in accessible surface areas of unfolding, ΔASA. It is not understood whether these correlations can be applied to larger proteins. In this work, to clarify the generality of these correlations and to find new insights into protein stability, we examined the urea-induced unfolding of pitrilysin and its C-domain (pitrilysin-Cd) from *E. coli*. Pitrilysin and pitrilysin-Cd exist as a monomer with 939 amino acid residues and 495 amino acid residues. They exhibit reversible unfolding against urea denaturation at some particular conditions. Urea-induced unfolding curves monitored by fluorescence and CD were analyzed according to a three-state model.

The m value and ΔC_p of pitrilysin-Cd are comparable to the values expected from its size. On the other hand, those of pitrilysin are quite smaller than the predicted ones. These results indicate that for large proteins, the correlations of m value and ΔC_p with protein size should be reconsidered. To construct a new correlation, ΔASA was divided into two parts, ΔASAnp derived from non-polar atoms and ΔASApol derived from polar atoms, because the composition of buried polar atoms increases in large proteins. This improves the correlations, but they should be improved more using other factors.

2P067 高圧力 NMR 法による staphylococcal nuclease Δ+PHS および Δ+PHS V66K 変異体の構造揺らぎの研究
Conformational fluctuation of highly stable form of SNase Δ+PHS and its V66K variant studied by high pressure NMR spectroscopy

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The structural and dynamic properties of a highly stable form of staphylococcal nuclease known as Δ+PHS and its V66K variant, which has a Lys side chain inside the main hydrophobic core were investigated using high-pressure NMR spectroscopy at pH 6 where the internal Lys66 is neutral. Examination of the pressure effects on the ¹H-¹⁵N HSQC spectrum showed significant shifts in both the ¹H and ¹⁵N frequencies at all residue sites, reflecting average changes in hydrogen bond distances and in ϕ and ψ torsion angles. Although the majority of the shifts are linear, some are significantly non-linear. The continuous shifts without loss of signal intensity indicate that these fluctuations are rapid on the NMR time scale (~ms). To treat quantitatively the non-linear shifts, we fit both ¹H and ¹⁵N shifts to a 2nd order polynomial. In both the ¹H and ¹⁵N dimensions, significant non-linear responses were observed for the variant, which are clustered in proximity to Lys66 in helix-1 and in the β-barrel. The linear shifts represent the conformational fluctuation within

the basic folded conformation, whereas the non-linear shifts are attributable largely to the increase in the population of low-lying excited state conformations. These results indicate that the insertion of the Lys side chain into the hydrophobic core significantly increased the conformational fluctuations (<ms) into low-lying excited states, in fair agreement with the conclusion from the H/D exchange NMR experiments.

2P068 ユビキチン変異体を用いた高エネルギー構造と機能の研究 High pressure NMR reveals significant shifts in populations of higher-energy conformers in ubiquitin variants

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The structure of a protein in solution takes not only the basic folded state but also higher energy states under physiological conditions. Previously, by using high pressure NMR spectroscopy, we found higher energy conformers (alternative form N₂ and locally disordered form I) in the functionally related homologues ubiquitin and ubiquitin-like proteins (NEDD8 and SUMO-2), and suggested a conserved higher energy conformer for their common functional strategy, the E1-E2-E3 cascade. To find a link of such low-populated higher energy conformers to ubiquitin function, we extended the approach into the ubiquitin variants. A breaking of the hydrogen bond or a replacement of Pro-Pro sequence to Ala-Ala in ubiquitin (K11A, E34A, Q41N, Q41A and P37A/P38A) brought about significant shifts in both the 1H and 15N frequencies at the E1 and E2 binding region of the proteins, indicating a large increase in the population of higher energy conformers. High pressure NMR experiments gave ΔG^0 and ΔV^0 values for the N₁-to-N₂ and N-to-I transitions of the proteins. In addition, we determined the binding constants of ubiquitin-interacting-motif UIM to ubiquitin and its variants. We will discuss the structure-function relationship among the ubiquitin variants in terms of the stability of higher-energy conformers.

2P069 Van der Waals replica-exchange method to overcome steric 1E1505 restrictions

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Effective samplings in the conformational space are necessary to predict the native structures of proteins. The replica-exchange method is one of the most well-known methods among the generalized-ensemble algorithms which realize effective samplings in the conformational space for biomolecular systems. However, the replica-exchange method has a difficulty for large systems that many replicas are needed to realize such effective samplings. In other words, huge amount of computation time is required in the replica-exchange method for the large systems.

In order to overcome this difficulty, we propose a new type of the Hamiltonian replica-exchange method, where the van der Waals radius parameter is exchanged although the temperature is exchanged in conventional replica-exchange method. By decreasing the van der Waals radii, which control spatial sizes of atoms, this Hamiltonian replica-exchange method overcomes the steric restrictions and energy barriers for biomolecule systems. Furthermore, the simulation based on this method escapes from the free-energy local-minimum states and realizes effective sampling in the conformational space of the biomolecule systems. We applied this method to an alanine dipeptide in aqueous solution and showed the effectiveness of the method by comparing the results with those obtained from the conventional canonical and replica-exchange methods [1].

Reference

[1] S. G. Itoh, H. Okumura, and Y. Okamoto, J. Chem. Phys. 132, 134105 (2010)

2P070 ストリング法によるアデニル酸キナーゼの構造変化解析 Capturing large-scale conformational transitions of adeny- late kinase using the string method

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Characterizing large-scale conformational transitions of proteins is an important issue in biophysics. Given a set of collective variables to describe the conformational changes, a minimum free energy path (MFEP) is defined as a transition path of maximum likelihood in these variables. However, it is not feasible to obtain the MFEP from brute force MD simulations because large free energy barriers in the collective coordinate space prevent accurate sampling of the conformational space within the available computer time.

To circumvent this problem, we propose to combine the recently developed "on-the-fly" string method (Chem. Phys. Lett. **446**, 182 (2007)) and principle component analysis (PCA), where PCs are used as relevant collective variables. In the string method, the MFEP is sampled concurrently by a set of replicas of the MD system. Each replica is attached to a particular value or "image" of the collective variable, providing the data to evolve this image. The coupling strength between images is weak enough to make the method trivially parallelizable. We have applied this string method to the large-scale conformational change of adenylate kinase from E. coli. We have checked the convergence of our new algorithm for this biomolecular system in explicit water, and MBAR (J. Chem. Phys. **129**, 124105 (2008)) was successfully applied to estimate the free energy along the path. We further discuss the comparison to the previous computational work and the biological implication of our result.

2P071 二次構造情報を取り入れた分子動力学法によるタンパク質立体 構造予測法の一般性の検証 Generality of the protein structure prediction method based on molecular dynamics simulation with secondary structure information

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Predicting the native structure of a protein from its amino-acid sequence is one of the most challenging problems in biophysics. In principle, the native structure can be found as the most populated structure in a canonical ensemble generated by molecular dynamics (MD) simulation. However, this approach immediately comes up against the following problems: (i) inefficiency of sampling, (ii) inaccuracy of the potential energy function, and (iii) vastness of the conformational space. To solve these problems, we developed a method that incorporates the secondary structure information into the MD simulation. This method is a kind of Bayesian inference; the prior distribution for the structure generated by the MD simulation is corrected by the secondary structure information. We used a smoothed delta functions as the likelihood, which is 1 if all the backbone dihedral angles of the residues that have secondary structures are within predefined ranges and 0 otherwise. In the MD simulation, the likelihood acts as the restraints for the backbone dihedral angles. We have shown that the native tertiary structure of a protein was accurately predicted as a structure that had the lowest free-energy by performing the MD simulation at a higher temperature with the restraints. In the present study, we explored the generality of this method by applying it to various proteins with different folds. Effects of choice of force fields, generalized Born models, and temperature regulation methods are also discussed.

2P072 βラクトグロブリンのフォールディング中間体類似状態の超遠 心分析 Ultracentrifuge analysis of folding intermediate analogues of βlactoglobulin

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It is widely known that larger protein collapses in the burst phase of folding and populates an intermediate with a certain amount of secondary structure. It is not fully understood whether hydrophobic collapse promotes the secondary structure formation or interaction of the secondary structure elements makes the intermediate to be compact. Equine β lactoglobulin (ELG) is a protein of 162 residues, which has 8-stranded β barrel fold. Intriguingly, the intermediate with a secondary structure including nonnative α helix was observed during burst phase of folding by CD stopped flow analysis. We investigated sedimentation velocities for an equilibrium analogue of the burst phase folding intermediate of ELG and its mutants, in which secondary structure elements was specifically disrupted by site-directed proline substitution. From the sedimentation coefficient and the partial specific volume, Stokes radius was estimated. Disruption of any secondary structure elements did not change Stokes radius, indicating that compact globularity of the intermediate is not a result of specific interaction of secondary structure elements. This result suggests that hydrophobic collapse in burst phase of protein folding can occur without secondary structures and consequently it promotes secondary structure formation.

2P073 NMR および CD 分光法を用いたマウスリポカリン型プロスタ 1E1520 グランジン D 合成酵素の熱変性中間体の解析 NMR and CD analysis of an intermediate state in the thermal unfolding process of mouse lipocalin-type prostaglandin D synthase