overall affinity for oxygen (P50), and cooperativity (Hill coefficient, n_{max}). In the presence of NaI, Hb showed two distinct behaviors depending on the concentration of this ionic chaotrope. At concentration of 0.1 M NaI, a drop in both K1 and K4 was observed. Increase in NaI concentration above 0.1 M produced a progressive increase in K1 values. However, as the concentration was increased to 1 M and above, oxygenation curves showed symmetry, suggesting the formation of functional dimers. Size exclusion chromatography confirmed the average molecular weight of the species being dimeric forms of Hb. The use of zwitterionic kosmotropes revealed two contrasting effects on the oxygen binding of Hb. Compared to native Hb, in the presence of increasing concentrations of 3-(1-piridino)-1-propane sulfonate, K1 values increased while K4 values remained constant. In the presence of dimethylbenzylammonium propane sulfonate, in addition to the effect on K1, K4 decreased producing consequently a drop in cooperativity. All of the above-mentioned effects strongly suggested that these solutes produced dimeric forms of Hb. However, contrary to the common belief, these dimers showed clearly allosteric properties, such as modulation of ligand binding and cooperative binding.

2P-071 溶媒和自由エネルギー計算によるタンパク質の osmolyte 効果の分子及び熱力学的基礎

Molecular and thermodynamic basis of osmolyte effects to proteins by solvation free energy calculations

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Biomolecules in cells function not in pure water but in a highly crowded, concentrated, non ideal solution environment. Therefore the usual treatments which make use of concepts from the ideal, infinite dilution solution limit are quantitatively inadequate for many biological problems. The effect of osmolytes and a multicomponent environment in general is to change the chemical potentials of the components, most notably that of the macromolecular solutes. Such changes result in differences in stability for conformations and oligomerization versus simple aqueous solutions. The effects can be quite significant for biological systems where common use is made of urea, TMAO, proline, sucrose, glycerol, etc.

We performed replica-exchange molecular dynamics simulations of a peptide in different solutions in order to explore the molecular basis of protein's stabilization/destabilization effects by osmolyte. We also calculated solvation free energy of different folded/unfolded peptide's conformations in order to examine the thermodynamic basis. Preferential solvation analysis showed that urea gathered around a peptide in excess, though TMAO was almost fair. Urea and TMAO tended to occupy similar regions around a peptide while water occupied different ones. We discuss the physicochemical origin of osmolyte effects through these molecular and statistical thermodynamic analyses.

2P-072 酵母プリオン系におけるプリオン株形成の分子機構

Molecular basis of prion strain phenotypes in the yeast prion [PSI⁺] system.

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The yeast prion state [PSI⁺] results from self-propagating aggregation of the translation termination factor protein Sup35. Transient overexpression of Sup35 N-terminal and middle domains (Sup-NM) leads to protein aggregation and de novo appearance of [PSI⁺]. Sup-NM also forms self-seeding amyloid fibers in vitro. Sup-NM amyloids formed at different temperatures adopt distinct, stably propagating conformations. Infection of yeast with these different amyloid conformations leads to different [PSI⁺] strains. In this study, we investigate the mechanism of Sup-NM different amyloids formation at different temperature. By using small angle X-ray scattering (SAXS), the existence of oligomers of Sup-NM was demonstrated at low temperature. There is significant correlation with the quantity of oligomer before amyloid formation and induced yeast phenotype, so oligomer is very important factor to decide the amyloid conformation. From SAXS measurements of proline scanning mutants, it was revealed that tyrosine residues in broad region are important to keep with the oligomeric structure, which is inconsistent with amyloid core region.

2P-073 NMRによるヒトカルシトニンアミロイド線維形成阻害機構の解明 Amyloid fibril inhibition mechanism of human calcitonin as studied by NMR spectroscopy

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Human calcitonin(hCT) is an amyloid forming peptide in concentrated aqueous solution, associated with medullary carcinoma of the thyroid. The fibril forming mechanism of hCT has been explained in terms of a two step reaction mechanism. The first step is a homogeneous association to form nucleus of the fibril and the second step is an autocatalytic elongation of the fibrils. The first nucleation process (k_1) is considered that the α -helices in the hCT monomers are bundled by hydrophobic interactions and consequently change to a nucleus. The second elongation process (k_2) is to elongate the fibril by associating a monomer of hCT molecule. In this study, three types of fibril inhibition mechanisms for hCT were investigated. First, Y12L/F16L/F19L-hCT was prepared and the fibrillation kinetics were examined. It turned out that the k_2 was significantly reduced in this mutant of hCT, while k_1 was not. Second, We have realized that charged amino acids such as Arg and Lys can also inhibit the fibril formation of hCT. In case of the addition of Arg or Lys, k_1 was significantly reduced, while k_2

was not. It is, therefore, explained that Arg and Lys may associate weakly with hCT in the solution, leading to prevent aggregation of hCT molecules in the nucleation process. Third, polyphenol compound curcumin was added in the hCT solution. In this case, hCT fibril formation was completely inhibited. This case, it is clarified that curcumin may strongly bind to hCT during fibril forming process and completely inhibit the nuclear formation.

2P-074 誘電緩和と分光によるネイティブ状態および酸変性状態の

Pseudomonas aeruginosa シトクロム c の水と解析

Hydration study of native and acid-unfolded cytochrome c551 of *Pseudomonas aeruginosa* by dielectric relaxation spectroscopy

Tetsuichi Wazawa(1)(2), Takashi Miyazaki(1)(2), Katsuhiro Kambe(1)(2), Takafumi Sonoyama(1)(3), Yoshihiro Sambongi(1)(3) and Makoto Suzuki(1)(2)(1:CREST, JST; 2:Graduate School of Engineering, Tohoku University; 3:Graduate School of Biosphere Science, Hiroshima University)

Protein hydration is an essential factor that governs the conformational stability and the folding mechanism. Dielectric relaxation spectroscopy (DRS) is one promising technique for analyzing protein hydration. *Pseudomonas aeruginosa* cytochrome c551 (PA cyt c) is a useful model to study protein folding, since its acid-unfolding process is known to be a two-state transition. In this study, we have examined the dielectric relaxation (DR) spectra of PA cyt c to investigate its hydration in the native and acid-unfolded states.

A pH titration of ferric PA cyt c monitoring the Q-band absorbance showed that the transition occurred with the pKa and Hill coefficient of 1.8 and 2.1, respectively, at 20.0°C. DRS measurement of ferric PA cyt c over a range of 0.2-26 GHz was performed at unfolding and native conditions (pH 1.1 and 3.5, respectively). A least squares analysis of the spectrum of PA cyt c solution was able to resolve the DR spectrum of a sphere region comprising only the hydration water and protein molecule. The DR spectrum of the hydrated protein was well simulated with the sum of two Debye functions with DR frequencies of ca. 0.3 and 5 GHz. Particularly, the DR at 5GHz should be attributed to a constrained water layer surrounding the protein. The analysis showed that the volume occupied by the constrained water increased by 1.7-fold upon unfolding, compatible with the general view of unfolded protein conformation. At present, we are investigating pH dependence of the DR spectrum of PA cyt c to study the folding mechanism.

2P-075 蛋白質の多様なフォールディング形態に対する多次元ランドスケープ理論

Multi-dimensional Landscape Theory of Diverse Folding Behaviors of Proteins

Kazuhiro Itoh and Masaki Sasai (Department of Computational Science and Engineering, Nagoya University)

Intense interest has been focused on how protein folding pathways are determined, how transition state ensembles are organized, and how these characteristics manifest in experiments of proteins. The energy landscape theory, which describes the statistical features of the free energy surface of protein conformational change, has provided a transparent view to these problems. Since a protein conformation is determined by a vast number of atomistic degrees of freedom, the free energy surface of protein conformation is intrinsically high dimensional. The energy landscape theory provides a guideline to project this high dimensional surface onto a few reaction coordinates. Especially, the one-dimensional representation of folding with a single reaction coordinate has been useful to describe the folding transition. In order to further analyze the folding mechanism, however, two or higher dimensional representation of free energy surface should become important.

We explain construction of the multi-dimensional surface of free energy describing diverse folding behaviors of single- and multi-domain proteins such as the multiple-state transition through intermediates, downhill folding, and multiple-pathway folding. Conditions that specify the various folding transitions and the instability of the folding behavior by changing parameters and mutation are discussed in terms of cooperativity and chain connectivity based on the native structure.

2P-076 SOD1 蛋白質の線維状凝集に関わる構造変化とその分子メカニズム

Molecular dissection of SOD1 aggregation and its structural rationale

Yoshiaki Furukawa, Kumi Kaneko and Nobuyuki Nukina. RIKEN, Brain Science Institute, Lab for Structural Neuropathology

Dominant mutations in Cu,Zn-superoxide dismutase (SOD1) cause a familial form of amyotrophic lateral sclerosis (fALS). Accumulation of insoluble SOD1 in affected tissues is a pathological hallmark found in fALS. Given that SOD1 is one of the most thermostable proteins, it is enigmatic how a soluble SOD1 protein forms insoluble aggregates. To become an active enzyme, an SOD1 polypeptide needs to be post-translationally modified: the copper/zinc binding and the intramolecular disulfide formation. These post-translational modifications increase the protein thermostability and possibly suppress the protein-protein interactions that eventually lead to the formation of insoluble aggregates. Indeed, we have recently shown that apo-SOD1 without disulfide is the only state for the formation of amyloid-like fibrillar aggregates (1). A role of a fALS mutation in the SOD1 aggregation is to impair either Zn binding, disulfide formation or both and lead to accumulation of the aggregation-prone, apo and disulfide-reduced SOD1 (1). A remaining issue to be clarified is how SOD1 changes its structure upon the fibrillar aggregation. In this presentation, based upon our recent

biochemical as well as immunochemical experiments, structural changes of SOD1 that occur upon its fibrillar aggregation will be discussed.

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2P-077 小さな球状蛋白質の熱容量と立体構造

Heat Capacity and conformation of small proteins

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Calorimetry experiments often assume that heat capacity changes of protein denaturation are dominated by hydration effect. In this study, heat capacities of bovine pancreatic trypsin inhibitor (BPTI) and Protein G are calculated by using extended scaled particle theory (XSPT). Temperature dependence of hydration free energy is used to calculate the heat capacities and entropies of each protein. Calculated values of heat capacity changes, ΔC_p , have shown that the protein has constant values in the range of 273-373K. In XSPT, the expression of hydration free energy has the term named C2, which has the same dimension as the square of the radius of the solute molecule. We have applied two different approximations to the expression of hydration free energy which is a functional of excluded volume through the concept of hypothetical scaling. New approximation is intended to treat interior cavities explicitly. Differences of ΔC_p values of BPTI between calculated and experimentally measured ones reduce 30% in the case of explicit treatment than before. In addition, the C2 term leads us to make a simple explanation of the existence of the convergence temperature of entropy changes (ΔS) by borrowing the concept of bilinear model proposed by B. Lee. The interior small cavities in BPTI increases the ΔC_p values 35% larger than those of Protein G, which has almost the same number of residues as BPTI.

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2P-078 大腸菌由来 pitrilysin の安定性解析

Analysis of the conformational stability of a giant protein, pitrilysin from *E. coli* Natsumi Ando, Atsushi Mukaiyama, Yuichi Koga, Kazufumi Takano, Shigenori Kanaya (Dept. Material & Life sci., Grad. School of Eng., Osaka Univ.)

To clarify the conformational stability of large proteins, we examined the urea- and heat-induced unfolding of pitrilysin from *E. coli*. Pitrilysin is a monomeric protein with 939 amino acid residues and its crystal structure has already been determined. Urea-induced unfolding was measured with CD at 220 nm and with fluorescence spectroscopy after excitation at 295nm. Heat-induced denaturation was studied with DSC. Pitrilysin exhibits reversible unfolding against both urea and heat denaturations, at some conditions, which indicates that even large proteins can unfold reversibly and pitrilysin is suitable for investigating the conformational stability of large proteins.

The fluorescence and CD equilibrium data were analyzed in combination according to a three-state model and the equilibrium populations of the native, intermediate, and unfolded state were estimated. The intermediate state is much accumulated at medium concentrations of urea (3M) in acidic and alkaline pH but only small amount of the intermediate was detected in neutral pH. The same results were obtained from DSC analysis. The DSC curves show a single thermal transition around neutral pH. In contrast, two independent transitions were observed in the acidic and alkaline pH ranges. Pitrilysin consists of two domains. These results suggest that the electrostatic interactions between the domains regulate the unfolding of pitrilysin. Two domains unfold cooperatively in the neutral pH and individually in the acidic and alkaline pH ranges.

2P-079 グルコアミラーゼのデンプン結合ドメインの安定性とリフォールディング過程に対するジスルフィド結合の効果

Influences of the disulfide bond of the starch-binding domain of glucoamylase on the protein stability and refolding

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The starch-binding domain of glucoamylase has a single disulfide bond. We attempted to analyze the effects of the disulfide bond on the protein stability and refolding.

Differential scanning calorimetric studies showed that the unfolding temperature $t_{1/2}$ of the disulfide-deficient mutant decreased by 10 degrees compared to the wild-type protein at pH 7.0. At $t_{1/2}$ of the wild-type protein, the mutant destabilized by 11 kJ mol⁻¹ in terms of the Gibbs energy change. It was shown that the mutant was stabilized in terms of enthalpy, but a higher entropy change overwhelmed the enthalpic effect, resulting in destabilization. The CD spectra and the thermodynamic parameters of the binding with β -cyclodextrin at 25°C suggested that the native structure of two proteins is essentially the same. The increased entropy change can be attributable to the increase in the disorder of the unfolded state of the mutant.

The mutant populated a kinetic intermediate by rapid temperature decrease from higher temperature. It was shown that this intermediate contained specific side-chain interactions, and bound with β -cyclodextrin, suggesting that the intermediate is highly-ordered and largely native-like. The CD spectra showed that the secondary structure was once disrupted during the transition from the intermediate to the native state. The intermediate is considered to be an

off-pathway one, misfolded state, that has to undergo unfolding on its way to the native state. The disulfide bond in the wild-type protein is likely to impede misfolding.

2P-080 分子体積揺らぎによる蛋白質の動力学的特性の解析

Dynamical Properties of Proteins as Analyzed by Molecular Volume Fluctuation

Kunitsugu Soda(1), Yasutaka Seki(1), Yuda Shimbo(1), Hiroki Matsumoto(1) and Jumpei Fujii(1). (Dept. Bioeng., Nagaoka Univ. Technol.)

According to the thermodynamic fluctuation-dissipation relation, analysis of the structural fluctuation of proteins gives us information on thermodynamic susceptibilities, essential parameters characterizing their functional capability. Based on the above, we have been studying the volume fluctuation of proteins and their structural elements using molecular dynamics simulation method. As a result of it, we found that five globular proteins examined show common dynamical properties as follows: The volume fluctuation of a protein molecule arises mainly from two types of internal motions; that is, the oscillatory motion with a center frequency near 1 THz in the intermediate frequency (IF) range and the diffusive motion in the low frequency (LF) range of less than 0.1 THz. At room temperature, the power spectrum of volume fluctuation decreases in inversely proportion to frequency in both LF and HF ranges, but with different exponents. While the oscillatory motion has higher power spectral density than the diffusive motion at temperatures lower than about 100K, the magnitude relation is reversed at room temperature. The correlation length of residue-volume fluctuation is shorter than 1 nm, which means that the volume fluctuation is significantly correlated only between pairs of not-covalently-bonded neighboring residues. Detailed molecular mechanism underlying the frequency dependence of the power spectral density will be discussed in the poster.

2P-081 タンパク質の内部運動による機能制御機構の実験的解明

NMR analysis on the protein dynamics and function relationship

Eiji Ohmae (1), Yuji Horiuchi (1), Kumihiro Gekko (1) and Shin-ichi Tate (1)

Protein structural dynamics and function relationship has been recognized as an essential aspect for exploring the functional mechanism of protein. In particular, the intrinsic protein structural fluctuation, ranging in the μ sec to msec time regimes, that is defined by each specific protein structure should have significant roles in the functional regulation, although it has not been clearly shown by experiments.

We have found some mutations in the loop regions in *E. coli* DHFR, dihydrofolate reductase, have significant reduction in the enzymatic activities, although the mutation sites are far from, appr. 30A, the active site of the enzyme. We thought that this "long-distance" effect should be caused by the disturbance in the concerted protein motion that includes both the active site and the distant mutation site. To explore the long-range effect of the loop mutation, we carried out the NMR spin relaxation analyses focusing on the protein dynamics in the time regimes from μ sec to msec. The results have shown that the mutation in the flexible loop has caused the significant modulation on the protein dynamics, which results also demonstrate that the flexible loop and the active site are moving in a concerted manner. The mutation in the loop, thus, disturbs the concerted motion, which results in a motional modulation of the distant active site. The present work shows the importance of the intrinsic internal motion in the enzymatic activity of the DHFR.

2P-082 生体分子機械の動きの特徴付け—歪み緩和過程の粗視化動力学計算による比較研究

Nonlinear Relaxation Phenomena in Coarse-grained Molecular Dynamics and Characterization of Motion in Bio-molecular Machines

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Large-scale conformational motion of bio-molecular machines including enzymes may be important for the machine functions. It still costs too much computation to reproduce such slow motion by detailed molecular dynamics simulations; coarse-grained models such as Go-models have been adopted.

We sought an easy and plausible way to characterize molecular motion. Previously, we studied relaxation phenomena in elastic network models (ENM; network of material particles and linear springs) starting from randomly chosen initial deformations [1]. As we pointed out, nonlinearity is significant for large conformational changes often seen in the machine cycle.

We found, however, that such nonlinear behavior varies according to species of molecules. In this work, we studied motion again in ENMs and their variants, and classified the motion by a profile of the elastic potential energy. For example, an elastic network model for KIF1A shows a sequence of conformational motion (step-by-step reconfiguration), and correspondingly there appears a plateau in the energy profile (i.e., the relaxation is strongly hindered). We also extended the model, allowing partial unfolding, to discuss the validity and limitation of these models. Relevance of the type of motion to possible effects of fluctuations (i.e., hydrodynamical fluctuations of the solvent [2]) is also discussed.

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