located in the Ca^{2+} binding site. In CML, the A-helix (protection factor ~ 50-500) was the most protected element in the MG state. These results show a rare example of the phenomenon wherein proteins that are very homologous to each other take different folding pathways.

3P-021 シーディングによるマウス PrP アミロイド線維の伝播 Propagation of mouse PrP amyloid fibrils by seeding

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Prion diseases are a group of fatal neurodegenerative diseases that include Creutzfeldt-Jakob disease in human as well as scrapie and bovine spongiform encephalopathy in animals. These disorders are caused by the conversion of native α -helical prion protein (PrP^{C}) into its pathological β -sheet rich isoform (PrP^{Sc}), and could be transmissible between distinct species. PrP^{Sc} particles composed of the same protein lead to phenotypically distinct transmissible states, in which PrP^{C} is able to misfold into multiple PrP^{Sc} forms. To obtain further insight into the propagation mechanism of PrP amyloid fibrils, we investigated the fibril formation of mouse PrP(23-231) by using distinct mPrP(23-231) seeds.

mPrP formed two types of aggregates, one was the amyloid-like- and the other was the aggregate-like fibrils when we observed by EM, and they were quite different in the intensity of ThT fluorescence and also in the CD spectrum. Amyloid-like fibrils had larger amount of β -sheet, and their ThT intensity was about twice higher than that of aggregate-like fibrils. We conducted the seeding experiments with those fibrils. As a result, the ThT intensity of fibrils formed by amyloid-like seeds was twice higher than those formed by aggregate-like seeds at a few rounds. This result implies that both fibrils with disparate conformations have a potential for the template-dependent propagation of amyloid fibrils. Seed-dependent fibril formation is somewhat similar to seed-dependent crystal growth at supersaturated state.

3P-022 一分子測定法を用いた緑濃菌由来シトクロム c の変性状態内の運動観察 Denatured state dynamics of Cytochrome c551 observed by single molecule fluorescence intensity measurements

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To understand how proteins fold into their native structures, it is necessary to observe a conformational dynamics of denatured proteins at the single molecule level. We therefore investigated the dynamic properties of cytochrome c551 from *Pseudomonas aeruginosa* (Cyt c551) by single molecule fluorescence intensity measurements. We first improved the single molecule observation system by introducing a new collection system of fluorescent photons based on a parabolic mirror. We next attached a fluorescence dye (ATTO) to 80th cysteine of Cyt c551 S80C mutant. We confirmed that the fluorescence intensity of the labeled protein increases significantly upon the denaturation, reflecting the lower FRET efficiency between ATTO and heme in the denatured conformation. Finally, we observed the folding dynamics of Cyt c551 in the presence of various concentration of denaturant by the single molecule measurements. We assigned the fluorescence intensity fluctuation with a time constant of ca. 70 ms to the conformational dynamics within the denatured state. The time constant becomes shorter at the lower concentration of denaturant. These conformational dynamics were interpreted to correspond to the dynamics between an expanded conformation and a compact conformation both in the denaturant state. Further experiments are underway to reveal the properties of the conformational dynamics in the denatured state.

3P-023 全原子モデルによる小球状タンパク質のフォールディングエネルギー地形: マルチスケールモデリング Folding energy landscape of small globular proteins at atomistic resolution revealed by multiscale modeling

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Energy landscape perspective has been helpful to understand protein folding dynamics. To compute energy landscape for specific proteins, broad-range of sampling is indispensable. For the purpose, we have been using coarse-grained (CG) protein model, which simplifies side-chain as "a sphere". As the result, side-chain packing information is severely weakened. Here, we combine this coarse-grained sampling with atomistic energy minimization to get the atomistic-resolution energy landscape of folding proteins. We sampled protein G and src-SH3 domain by fragment assembly, a CG SimFold energy function, and multi-canonical ensemble method. We re-constructed all-atom side-chains to these samples, which were then put into all-atom molecular dynamics (AMBER) simulation of short time to get local minima in atomistic landscape. Some miss-folding structures found by the CG model, which have the energy comparable to native structure's energy, turned out to have much higher energy in the re-constructed atomistic-resolution energy landscape. As the results, atomistic-resolution energy landscape looks prominently more funnel-like, comparing with that by the CG model.

3P-024 論理的創薬法を用いた抗がん剤候補化合物の開発 Novel therapeutic compound for Colon Cancer discovered by a rational drug design

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p53 is a tumor suppressor protein and its activation causes the apoptosis of cancer cells. In half of cancer patients, p53 is inactivated. Activated p53 binds with the specific sites of DNA, and intervene in the normal cell cycle. p53 is a known to be natively unfolded protein, and it folds upon binding with DNA. However, mutant p53 can no longer bind with DNA or form correct bound conformation and consequently it can not interrupt the cell division. If we can artificially design a small compound which can act as a chemical chaperon capable of stabilizing the folded p53 conformation, it might be able to inhibit the proliferation of cancer cells. The purpose of this study is to find the small molecules that can assist the folding p53 and activate it, thereby possessing the potential activity to decrease the tumor growth. ZINC database (~about 600,000 compounds) was used for docking simulations. Flexibility of compounds were determined automatically using AUTOTORS, which is a program of the AutoDock package based on the docking energy. We finally extracted 70 compounds. Then, we examined the effect of 70 compounds using human colon cancer cells, HCT116. We adopted the MTT assay method to examine the real effect of those compounds. We conducted in vivo experiment using nude mice, and found one compound, termed GJC29 has a strong suppressive effect on the cancer proliferation. Moreover, we confirmed the binding of compound to p53 by SPR. Thus the rational drug design strategy is considered to be quite useful for the discovery of chemical chaperons.

3P-025 古細菌型ロドプシンを発現した大腸菌の膜生理学 Membrane Physiology of *Escherichia coli* Expressing Archaeal Type Rhodopsins

Kunio Ihara (Center for Gene Research, Nagoya University)

Overexpression of membrane protein using heterologous host such as *E. coli* is essential for physicochemical or structural studies. In this end, the yields of the objective membrane protein should be of great interest and the physiological response to overexpression membrane proteins in *E. coli* has not been well studied. However, it is important to understand the physiological response of membrane protein overproducing *E. coli* to improve the quality and yields. We have used archaeal type rhodopsins, which have 7 transmembrane domains, as model membrane proteins and expressed in *E. coli* using arabinose regulated expression systems. Archaeal rhodopsins were all quantitatively expressed in response to the concentration of arabinose. However, the extents of the functional folding in the membrane, which were assessed by the change of the color after the addition of retinal, were very different by expressing proteins (aR, bR, cR, dR and xR). Overexpression in the presence of 1.0% arabinose lead to the filamentous phenotype due to incomplete cell division in all cells. Light pH changes or ATP synthesis were observed in all except for bR and xR expressing cells. These observation indicated that aR, cR, and dR were expressed in the functional state, but bR and xR were not. Because these two archaeal rhodopsins were expressed in the membrane, only the final folding processes maybe remain in the half way. We will report the effect of co-expression of membrane protein chaperons YidC or DnaK for the functional expression of bR or xR.

3P-026 Proteomic Modeling of Protein Conformational Stability using Amino Acid Sequence Autocorrelation Vectors and Support Vector Machines Proteomic Modeling of Protein Conformational Stability using Amino Acid Sequence Autocorrelation Vectors and Support Vector Machines

Michael Fernández, Akinori Sarai (Department of Bioscience and Bioinformatics, Kyushu Institute of Technology)

The conformational stability of more than 1500 protein mutants was modeled by a proteomic approach using amino acid sequences autocorrelation vector (AASA) formalism [1]. 48 amino acid/residue properties selected from the AAindex database weighted the AASA vectors. Genetic algorithm-optimized Support Vector Machine (GA-SVM), trained with subset of AASA descriptors, yielded predictive classification and regression models of unfolding Gibbs free energy change. Function mapping and binary SVM models correctly predicted about 50% and 80% of free energy variances and signs in crossvalidation experiments, respectively. Test set prediction showed adequate accuracies about 70% for stable single and double point mutants. According to the optimum model, conformational stability depended on autocorrelations at medium and long ranges in the mutant sequences of structural, physico-chemical and thermodynamic properties related to protein hydration process. A preliminary version of the predictor is available online on the World Wide Web at http://gibk21.bse.kyutech.ac.jp/llamosa/ddG-AASA/ddG_AASA.html [1] Proteins 67, 834, 2007.

3P-027 蛍光相関分光法および蛍光寿命イメージング顕微鏡を用いた変異型 SOD1 の凝集体形成と脱凝集機構の解析 Aggregation and disaggregation analysis of mutant SOD1 using fluorescence correlation spectroscopy and FLIM-FRET.

Akira Kitamura (1), Noriko Inada (2), Gen Matsumoto (3), Richard I. Morimoto (4), Hiroshi Kubota (6), Kazuhiro Nagata (5) and Masataka Kinjo (1). (1: Lab Mol Cell Dynamics, Hokkaido Univ Faculty of Advanced Life Science; 2: Nara Inst for Science and Technology; 3: BSI, RIKEN; 4: Northwestern Univ; 5: Inst Front Med Sci, Kyoto Univ; 6: Akita Univ)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons controlling muscle movement and strength. Each type of FALS is caused by mutations in a specific gene, and type I FALS patients undergo mutations in the Cu/Zn-superoxide dismutase (SOD1) gene. Most FALS-linked mutations render intramolecular disulfide bond reduction and accelerate proteasome-dependent degradation. Recent studies implicate the disulfide-reduced monomer as the common aggregate-prone neurotoxic intermediate of mutant SOD1 proteins. Although aggregate formation was not observed in the mutant SOD1-expressing cells under normal culture conditions, formation of aggregates-like structure were observed after treatment with the proteasomal inhibitor MG-132. Intriguingly, this aggregate-like structure disappeared after removing proteasome inhibitors, suggesting that HeLa cells have disaggregation activities. Fluorescence correlation spectroscopy analysis of cell lysate indicated that mutant SOD1 forms disulfide bond-linked soluble oligomers after MG-132 treatment and that these oligomers disappear after the removal of MG-132, probably due to disassembly of the oligomers. FLIM-FRET analysis revealed initial aggregate formation is widely occurred in cytoplasm. In poster presentation, we would also like to present results of our on-going analysis including FCCS experiments.

3P-028 芳香族アミノ酸変異による近紫外円二色性スペクトル解析

Analysis of near-UV circular dichroism spectrum by the aromatic residue substitution

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Equine β -lactoglobulin (ELG) has one tryptophan (Trp) and five tyrosine (Tyr) residues, which would contribute to the near-UV circular dichroism (CD) spectrum in the native state. To investigate contribution of each Tyr residue to the near-UV CD, mutant proteins in which each Tyr residue was replaced with phenylalanine residue (Y42F, Y86F, Y99F, Y102F and Y121F) were prepared and their CD spectra were measured under the conditions where ELG is in the native state. Y42F, Y86F and Y121F show a little change in the near-UV CD spectrum. Y121F also shows a change in the far-UV CD spectrum, suggesting that Y121F substitution may induce a structural change. Y102F shows decreased CD in the near-UV CD spectrum but does not show any significant change in the far-UV CD spectrum, suggesting that Tyr102 positively contributes to the near-UV CD spectrum in the native state. Y99F does not refold, suggesting that Tyr99 has an important role for folding and stability of ELG. To investigate structural change of each mutant in more detail, ^1H - ^{15}N Hetero-nuclear Single Quantum Coherence (HSQC) spectra are measured. The results will be reported.

3P-029 リゾチームの構造安定性に及ぼすシクロデキストリンの効果

Effects of Cyclodextrins on Thermal Stability and Conformation of lysozyme

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The conformational stability of protein is expressed as the difference of Gibbs energy between folded and unfolded states of the protein. To clarify the mechanism of protein folding, it is very important to know the conformational and thermodynamic properties of the two states. Cyclodextrin (CD) is a family of cyclic oligosaccharides composed of several glucoses and is able to include hydrophobic molecule as an alkyl group in the interior. Globular protein consists of hydrophobic and hydrophilic amino acids, and the hydrophobic amino acids are gathered preferentially inside of the protein for escaping contacts to solvent (water) at folded state. It is expected that CD molecule can include hydrophobic amino acid and/or hydrophobic part of protein especially at unfolded state. In this study, to clarify effects of CD on the stability and conformation of protein, thermal denaturation of lysozyme were measured in some kinds of CD aqueous solutions at various concentrations by DSC, viscometer, and circular dichroism. Thermal stability of lysozyme was monitored with a high sensitivity differential scanning calorimeter, MicroCal MC2, at a scanning rate of 1 K/min. Thermal denaturation temperature, calorimetric enthalpy change, and half-value width were calculated with a program attached to this instrument. Viscosities of solutions were measured using a heart-shaped viscometer in our custom-made thermostat that was maintained at various temperatures. The results will be discussed.

3P-030 PQBP-1の天然変性領域とスプライシング複合体 U5 の構成要素である U5-15kD との相互作用

A natively unfolded region of PQBP-1 interacts with U5-15kDa, a component of a splicing factor complex U5

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The polyglutamine tract-binding protein-1 (PQBP1) is ubiquitously expressed but is particularly abundant in the central nervous system. PQBP-1 has several functional domains, including a WW domain which binds to proline-rich motifs in other proteins, and a C-terminal domain responsible for the interaction between PQBP-1 and a pre-mRNA splicing factor complex U5 involved in the spliceosome. The C-terminal domain of PQBP-1 binds to the splicing factor complex U5 via U5-15kD, which is a component of the splicing factor complex U5. Mutations of PQBP-1, which are known to cause X-linked mental retardation (XLMR) accompanied by microcephaly at a high frequency, lead to total or partial deletion of the C-terminal domain of PQBP-1. An in vitro

binding assay between GST fusion XLMR-related PQBP-1 proteins and U5-15kD showed that XLMR-related PQBP-1 proteins lose the affinity for U5-15kD. We further studied the interaction between the C-terminal domain of PQBP-1 and U5-15kD. NMR studies indicated that PQBP-1 binds to U5-15kD by using a natively unfolded region, which is located in the C-terminal portion of the C-terminal domain. Chemical shift perturbations indicated that the PQBP-1 binding site on U5-15kD was mapped to the first helix and the fourth loop of U5-15kD. Furthermore, alanine-scanning mutagenesis revealed that Tyr245 and Asn255 are particularly important for the interaction with U5-15kD.

3P-031 両親媒性ポリマーと無機塩を用いたタンパク質リフォールディング

A refolding method to combine amphiphilic polymer and inorganic salts

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Polyethylene glycol (PEG) is one of the most widely used polymers for stabilization of protein in aqueous solution. PEG is, however, not enough to improve refolding yield of protein. We found the synergistic effects PEG with inorganic salts on protein refolding using hen egg lysozyme as a model. For examples, PEG400 (100 g/L) and NaSCN (1.5 M) increased the refolding yield five-fold (>95%) higher than the absence of additive (~20%), while only PEG400 (100 g/L) increased the refolding yield by two-fold (~40%). PEG and salting-in salts, such as iodide salts and thiocyanate salts, dramatically suppressed aggregation during oxidative refolding of reduced lysozyme. Solubility of reduced protein and amino acids and refolding kinetics of lysozyme indicated that PEG and salting-in salts synergistically inhibit nucleation-dependent aggregation of reduced protein.

3P-032 ポリアリルアミンによるリゾチームとリボヌクレアーゼAの加熱失活抑制

Poly(allylamine) prevents heat-induced inactivation of lysozyme and ribonuclease A

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Aggregation is a serious problem in biotechnology and pharmaceutical application of proteins. An approach to prevent protein aggregation is the addition of polymers in protein solution. We found that poly(allylamine) (PAAm), which is polyelectrolyte containing amino groups, dramatically prevented thermal inactivation of hen egg white lysozyme and bovine pancreas ribonuclease A (RNaseA). Almost all of lysozyme and RNaseA in sodium phosphate buffer (pH 7.0) inactivated by heat treatment at 98 °C for 10 min, while in the presence of 5 mg/ml PAAm, the residual activity of proteins was exceedingly maintained after the same heat treatment. PAAm was well functioned at a molecular weight of 1,000, 5,000 and 15,000. Amino groups of PAAm may interact with hydrophobic amino acids of proteins and decrease in the probability of protein-protein interaction due to the electrostatic repulsion. PAAm is a new type of materials which can prevent the aggregation and inactivation of heat-labile protein.

3P-033 フォトンカウンティングヒストグラム法と蛍光相関分光法を用いた $\alpha\beta$ 多量体形成の解析

Analysis of Ab oligomer formation by photon counting histogram (PCH) and fluorescence correlation spectroscopy (FCS)

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Amyloid beta (Ab) is 4 kDa peptide which is thought to form aggregation and cause Alzheimer disease (AD). Recently, it has been suggested that soluble Ab oligomers, are the causative agent of AD since such oligomers are more cytotoxic than fibrils. However, the mechanism how soluble oligomers are produced is still unknown. In this study, we analyzed formation of Ab oligomers at a single molecule level using photon counting histogram (PCH) and fluorescence correlation spectroscopy (FCS). Using PCH method, the number of protomers in oligomers and concentrations are obtained from histograms of photons from fluorescent molecules diffusing through the confocal volume. Using FCS method, the diffusion constant of molecules passing through the confocal volume can be obtained from fluorescence fluctuation using the same confocal optics with PCH. Combining the results from these experiments, the concentration distribution of oligomers can be calculated. Soluble Ab oligomers were formed using FITC-labeled Ab peptide and were analyzed by PCH. Ab monomer and tetramer were observed in the solution. The average diffusion constant of Ab oligomers (130 $\mu\text{m}^2/\text{sec}$) was smaller than that of Ab monomer (160 $\mu\text{m}^2/\text{sec}$), supporting the result of PCH. Detailed analysis of formation of Ab oligomer such as ADDL using these techniques is in progress.

3P-034 サツマイモ由来 Purple acid phosphatase 活性中心のハイブリッド DFT による電子状態解析

Hybrid DFT study on the electronic structure of active site of sweet potato purple acid phosphatase

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