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

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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 Proteometric Modeling of Protein Conformational Stability using Amino Acid Sequence Autocorrelation Vectors and Support Vector Machines

Proteometric 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 proteometric 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.

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