for the rapid folding.

In our poster, we will further discuss contributions of the α -helix intermediate of β -lactoglobulin in folding process.

3P-008 β 2-ミクログロブリンとの比較による免疫グロブリン軽鎖 C_L のアミロイド線維形成能の評価

The ability of fibril formation of the constant domain of immunoglobulin light chain in comparison with β 2-microglobulin.

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Immunoglobulin light chains are the precursor proteins of AL-amyloidosis. It is believed that the variable domain of the light chains is important for the formation of amyloid fibrils, and the role of the constant domain (C_L) has not been emphasized in the study of the fibrillation. However, C_L was also found in AL-amyloid deposits extracted from the patients, implying that C_L forms amyloid fibrils and contributes to AL-amyloidosis. The purpose of this study is to examine the ability of C_L to form fibrils at neutral pH.

The amyloid fibrils of C_L were formed by the two ways using, ultrasonication and agitation. Fibrillations were confirmed by thioflavin T (ThT) fluorescence, electron micrograph and CD. These results were compared with those of β 2-microglobulin (β 2-m) under the same conditions. β 2-m is the major component of amyloid deposits of dialysis-related amyloidosis, and the tertiary structure of β 2-m has high similarity with that of C_L . Interestingly, the different propensity was obtained between ultrasonication- and agitation-induced fibrillations. ThT fluorescence of C_L was higher than that of β 2-m in the agitation-induced fibrillation, but opposite result was obtained in the ultrasonication-induced fibrillation. In addition, the morphology of the fibrils differed depending on the solvent conditions, which was indicated by electron micrograph. We will discuss the distinct abilities of fibril formation in relation with the structural properties of these proteins.

3P-009 構造変化を伴う 2 量体形成と空間サイズ効果

Dimer formation with conformational change and effect of confinement: Molecular dynamics simulation

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Protein conformation is often different in its monomer and in a complex with other proteins; for example, domain-swapped oligomer, amyloid fibril and intrinsically disordered proteins which are flexible and can interact with multiple partners depending on the conditions. Oligomer formation process may depend on stability of monomer and of oligomer. Proteins are often confined in narrow space *in vivo*, and this confinement may affect their stability. We investigate the dimer formation process showing such conformational change using molecular dynamics simulation. So-called the dual-Go model[1], which is a protein model in a coarse-grained level, confined in a simulation box is used to reproduce a conformational change of monomer. We focus on the entropic effect on the dimer stability and dimerization dynamics by changing the size of the simulation box. [1] F. Takagi and M. Kikuchi, Biophys. J. vol. 93, pp.3820-3827 (2007)

3P-010 重水素交換法を用いたアミロイド線維伸長反応機構の解析

Analysis of the amyloid fiber extension mechanism using H/D exchange.

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The deposition of amyloid fibrils is associated with over 20 degenerative diseases. Dialysis-related amyloidosis is one of such diseases, and is commonly developed by patients undergoing long-term haemodialysis. The major component of the fibrils is β 2-microglobulin (β 2m). In the patients, β 2m is not degraded and forms amyloid fibrils, depositing in joints. It is considered that amyloid fibrils extend by monomer assembly on the fibril end, but the detail of the extension mechanism has not been understood yet. For amyloidosis treatment, it is necessary to clarify molecular extension mechanism of amyloid fibril and to determine which residue of monomer interacts with amyloid fibril end. However, amyloid fibrils cannot be observed directly using solution NMR because their molecular weight is too high. Therefore, in this study, we measured hydrogen/deuterium (H/D) exchange of monomer in the presence of the fibrils using solution NMR.

First, we determined our measurement conditions in which monomers and amyloid fibrils coexist by changing pH and the salt concentration. Next, we measured H/D exchange under determined conditions for exchange rate constants. Then, we observed no protection site in the absence of fibrils, but we observed protections in some residues in the presence of the fibrils. It is considered that these protections arose from interactions between monomers and fibril ends. We will discuss the amyloid fiber extension mechanism.

3P-011 ブラウン動力学／アンブレラサンプリングによる自由エネルギー計算法の開発

Development of the free energy calculation method using a Brownian Dynamics/Umbrella Sampling

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<Introduction> The protein-protein interactions seem to occur in millisecond time order. Thus, it is required to reduce the computational amount to reproduce such a long time required phenomenon in computer. For this purpose, we have developed a Brownian dynamics simulation to reduce the computational amount, in which, the solvent molecules are dealt with a continuous matter. Recently, we introduced an umbrella sampling method in this Brownian dynamics simulation and applied it to several 21-residue alanine-based peptides. <Method> Amber 91 force field was adopted for describing the intramolecular interactions in peptide. Effects of the solvent molecules on the structure of the peptide were included as the Distant-dependent Dielectric Constant model (DD), solvent-accessible surface area model (SA), and Effective Charge model (EC). The umbrella potential was applied between two terminus of the peptide. <Result and Discussion> Arginine-mixed peptide (Ace-A5(AAARA)3-Nme) had higher helical propensity than alanine-only peptide, which is compatible with the previous molecular dynamics simulations. The helical structures were stabilized by the electrostatic and van der Waals interactions overcoming the disadvantages in solvation energy from the NH and CO groups of the backbones. The calculated free energies are compatible with several previous experimental reports concerning with similar kinds of peptides, indicating that the umbrella sampling method makes the samplings across the broad phase space possible in our Brownian dynamics.

3P-012 分子動力学シミュレーションによる staphylococcal protein A の B domain のフォールディング機構の研究

Folding simulation of the B-domain of staphylococcal protein A

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The B-domain of staphylococcal protein A is a 46-residue, three-helix bundle protein. Although folding mechanism of this protein has been studied experimentally and theoretically by many researchers, detailed mechanism of its folding is still a matter of controversy. It has been shown that the L23G mutant is predominantly in a denatured state by experiment. To study how the mutation affects the folding of this protein, we compared the MD trajectories between the wild type and the L23G mutant, which were produced by constant-temperature simulations at 400 K with weak restraints on the backbone dihedral angles of the residues that form α helices in the native structure. The time of staying near the native state (C α RMSD from the NMR structure being less than 2.0 Å) of the mutant decreased to 4% of the time of the wild type. Since glycine can equally adopt positive and negative ϕ dihedral angles, its greater flexibility might affect the folding process. To verify this hypothesis, we carried out an MD simulation of the L23G mutant imposing additional restraints on the ϕ dihedral angles of G23 so as to have negative values throughout the simulation. However, the time of staying near the native state decreased to 1% of the time for the wild type. This result suggests that the lack of the interactions in which the side-chain of L23 is involved is a primary reason why the L23G mutant fails to fold.

3P-013 β 2 ミクログロブリンの天然状態におけるプロリン異性化反応の解析

Analysis of native-state prolyl isomerization of β 2-microglobulin

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β 2-microglobulin (β 2m), the light chain of the major histocompatibility complex type I, is found as a major component of amyloid fibrils formed in dialysis-related amyloidosis. The intermediate accumulated under acidic conditions is considered to be an amyloid precursor. However, it remains unclear how the folded β 2m forms amyloid fibrils under native conditions. To gain the insight into the amyloidogenesis of this protein under native conditions, we investigated the kinetic refolding of β 2m by stopped-flow fluorescence spectroscopy in combination with double jump techniques. Direct- and interrupted- unfolding and refolding experiments demonstrated that the slowest phase of refolding reaction was caused by the *cis/trans* isomerization at Pro32, which occurred in the folded state, and that *trans* isomer of Pro32 was present at equilibrium after folding reaction was completed in contrast to the *cis* form in the crystal structure. These results mean that the conformation of β 2m is heterogeneous even under native condition. To obtain further evidence for the conformational heterogeneity of folded β 2m, we used NMR spectroscopy and measured HSQC and NOESY-HSQC spectra. The chemical shift differences between *cis* and *trans* isomers were observed for His31 backbone amide, indicating that prolyl isomerization affects the local conformation around Pro32. Our results suggest that folded β 2m with *trans* Pro32 is involved in the amyloid formation under native conditions.

3P-014 四本鎖コイルドコイル GCN4-pLI に対する圧力効果

Pressure effect on the α -helix structure of coiled coil peptide GCN4-pLI

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Thermal stability of an α -helix has been extensively investigated using model peptides. However, understanding of pressure stability of an α -helix is still insufficient. Previously, we investigated pressure stability of GCN4-p1, which is a prototype of the α -helical coiled coil structure consisting of two amphipathic