1P-027 Nーメチル化ペプチドによるAβ線維化阻害

Inhibition of the fibrillization of amyloid β-peptide by its N-methylated fragments

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Alzheimer's disease is the most common type of dementia and a pathological hallmark of the disease is the deposition of senile plaques in the brain. The main component of the plaque is fibrillar aggregates of amyloid β-peptide (Aβ), which typically consists of 40 amino acid residues ($A\beta_{1-40}$). In the Aβ fibril model, the peptide forms β-strands in the middle (residue 12-24) and C-terminal (30-40) regions, and the β-strands are hydrogen bonded with the corresponding β-strands of adjacent molecules by using the main chain N-H and C=O groups. Accordingly, methylation of the N-H group is expected to inhibit the fibrillization of Aβ. In this study, we have examined the inhibitory effect of N-methylated analogs of the peptide representing the C-terminal β-strand region.N-methylation was made at one (L34), two (L34V36, L34G38), or three positions (L34V36G38) of the 11-mer sequence ^{30}AIIGLMVGGV^{40}V. $A\beta_{1-40}$ (50 μM) was incubated at 37 C in Tris-HCl buffer (pH 7.4, 50 mM) containing 150 mM NaCl in the absence and presence of the N-methylated peptide. The fibrillization reaction was monitored by measuring the fluorescence intensity of thioflavin T added to the sample taken from the incubation tube.The inhibitory effect was found to be strongest for the peptide containing only one N-methylated amide group at L34. The peptides methylated at additional one or two sites were unexpectedly less effective. This observation implies that N-methylation not only hinders intermolecular hydrogen bonding but also decreases the affinity of the peptide to $A\beta_{1-40}$.

1P-028 キメラβ-lactoglobulinの構造研究

A structual study of β-lactoglobulin chimeras

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β-lactoglobulin (LG) is composed of nine β-strands (A-I strand) and one α-helix. Eight out of the nine strands (A-H) form a β-barrel structure. Under physiological conditions, equine LG (ELG) is monomer, whereas bovine LG (BLG) exists as a dimer in which the off-barrel I strand forms an intermolecular β-sheet. Recently, the 3D structure of ELG was determined by NMR and revealed that the structure in the I strand region differs from that of BLG.To understand the reason why the structure in the I strand differs between two homologous proteins, we constructed several chimeric proteins. The mutants ImELG and ImBLG were constructed by substituting the amino acid sequence of I strand of BLG and ELG for those of ELG and BLG, respectively. Furthermore, Gyuba was constructed from the amino acid sequences of secondary-structured regions of BLG and those of loop regions of ELG. ImELG and ImBLG are monomeric, whereas BLG and Gyuba are dimeric. The crystal structure of Gyuba was determined at 2.0 Å resolution and showed that the backbone structure of Gyuba is closely similar to that of BLG, suggesting that the amino acid sequences in the loop regions were not important for the 3D structure of BLG. The structures of ImELG and ImBLG were investigated with NMR. HSQC spectrum of ImELG was similar to that of ELG. In contrast, HSQC spectrum of ImBLG was significantly different from that of BLG. These results suggests that I strand sequence of BLG can be adaptable to ELG structure whereas, I strand sequence of ELG may not be adaptable to BLG structure.

1P-029 actin capping protein/V-1(myotrophin)複合体のX線結晶構造

Crystal structure of actin capping protein in complex with V-1 (myotrophin)

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Actin capping protein (CP), a highly conserved heterodimeric protein, binds tightly to the barbed end of actin filament. CP is known to regulate a variety of cellular actin structures such as development of branched meshwork in lamellipodia and formation contractile ring at cytokinesis. We have previously reported the crystal structure of CP and an EM structure of the CP-actin filament complex. Based on the structural analysis, C-terminus region of α subunit of CP is thought to play important role for the interaction to actin filament, and further biochemical assays supported this idea. At present, number of factors are known to regulate barbed end capping activity of CP. V-1 (myotrophin) is a small ankyrin repeat protein implicated in several processes such as stimulation of protein synthesis in myocyte and synapse formation in neuronal cells. V-1 binds directly to CP and inhibits its ability to cap barbed end. In this study, we report the crystal structure of CP/V-1 complex and reveal the molecular mechanism of how V-1 regulates the capping activity of CP.

1P-030 分子動力学シミュレーションを用いた大腸菌機械受容チャネルMscLの張力受容部位の解析

Analysis of Tension Sensing Sites of E-coli Mechanosensitive Channel MscL Using Molecular Dynamics Simulations

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The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer α-helices and gated by tension in the membrane. 3D structure of MscL was resolved and its gating properties have been analyzed. Furthermore, some models for its gating process have been proposed based on Molecular Dynamics (MD) simulations. However, as those models do not apparently include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. In this study we performed MD simulations under a tension generated in the membrane. Upon tension generation, Phe78 in the outer helices, was dragged by lipids, leading to a tilting of the helices. Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor of MscL. Another outer helices amino acid Lys97 near the cytoplasmic surface also had a strong interaction with lipids when channel is closed, but this interaction gradually weakened as MscL opens, suggesting that Lys97 may not work as a tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. As tension increases, the crossings move toward the cytoplasm associated with an expansion of the constricted part. A calculated change in the interaction energy at the crossings during pore expansion was found to be consistent with the experimentally obtained activation energy for channel opening.

1P-031 分子間水素結合ネットワークの探索に基づく新しいタンパク質ドッキング計算法

Novel scheme for protein docking based on informatical complete search of intermolecular hydrogen bond networks

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Since protein-protein interactions play crucial roles in biological processes, structural analyses of such complexes have been one of the most crucial subjects in biological sciences. However, a lot of protein complexes are still escaped from structural determination; actually, for weakly interacting complexes, even the crystallization is a knotty issue. Thus, development of computational algorithms for protein docking is expected as an alternative to compensate such difficulties of experiments. In this study we have developed a novel scheme for the first phase of protein docking simulations, i.e. the rigid body docking calculations. First, we search for all of intermolecular/interfacial hydrogen bond networks (IHBNs) by an informatical technique, which is based on a clique detection algorithm in graph theory, within a framework of rigid body docking. Then, to identify a correct structure among ones generated based on the complete IHBN search, three steps for selection are sequentially carried out; in particular, the third step is most crucial: It has been found that ASA is quite effective to identify complexes close to experimental ones in our scheme.Our scheme has been tested using five known complexes, i.e. 1DXG, 1OHZ, 4HTC, 2SNI and 2PTC (PDB codes). As a result, it has been indicated that our scheme can predict complex structures within 2 Å of RMSD from crystal structures for all targets; this result show the highest accuracy of predictions, compared with conventional protein docking programs available, such as ZDOCK and BiGGER.

1P-032 協同運動増幅法を用いた効率的なタンパク質構造空間探索分子動力学シミュレーション

Molecular dynamics simulation with amplified collective motions method for extensive sampling in conformational space of proteins

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In recent years there has been an increasing use of theoretical methods, especially molecular dynamics (MD) simulations, to gain insight into structure-function relationships in proteins. Although we have progressed to tens of nanoseconds for the MD simulations of proteins with the improvements in computer power and algorithms, this time scale is still too short to observe many important protein processes, such as slow conformational changes, ligand binding, and protein folding/unfolding. To extent the capability of atomic simulations, various methods have been proposed. For example, multicanonical ensemble and replica exchange methods, adaptive umbrella sampling, conformational flooding and chemical flooding, essential dynamics simulation, and amplified collective motions (ACM) method have been proposed. Among them, the last three are based on collective coordinates. The use of collective coordinates has become an important technique to extract relevant motions from simulation results.In this study, we used ACM in combination with MD simulation for extensive sampling in conformational space of proteins. In ACM, the velocities of atoms are coupled in different subspaces with different thermal baths, the essential subspace (spanned by the limited number of slow collective modes) coupled to higher temperature and the remaining coupled to normal temperature bath. By ACM, it would be expected that the essential subspace can be sampled adequately and large amplitude motions of the protein can be observed during a simulation.

1P-033 マグネシウムトランスポーターMgtE細胞質ドメインのX線結晶構造解析

Crystal structures of the cytosolic domain of the Mg^{2+} transporter MgtE

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