2P-083 Trp 蛍光と H/D 交換-NMR 法をもちいた β_2 ミクログロブリンの Amyloid 線維形成反応中間体の解析

Monitoring the fibrillation intermediate of β_2 -microglobulin by Trp fluorescence and H/D exchange-NMR

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Protein misfolding occasionally leads to the formation of supramolecular assemblies known as amyloid fibrils, the deposition of which is associated with over 20 degenerative diseases. One of the most unique and essential properties of amyloid fibrils is their template-dependent growth, which underlies the propagation of the original structure of amyloid fibrils, and consequently, of pathology of amyloidosis. Identifying the molecular mechanisms of the fibril growth is an important issue to design effective strategies for the inhibition and regulation of amyloidosis.

In this study, we have investigated a monomer-fibril complex of β_2 -microglobulin formed transiently during the fibril growth by using Trp fluorescence and hydrogen/deuterium (H/D) exchange-NMR methods. When we analyzed kinetics of the seed-dependent reaction at different concentrations of seeds by using Trp fluorescence, we observed the accumulation of the fibril-monomer complex in the early stage of the reaction at excessive concentrations of seeds. Mutational analysis suggested that the region around Trp60 is one of the possible interfaces with the fibril. Furthermore, we have developed H/D exchange of the fibril-monomer intermediate. H/D exchange was performed under the accumulating condition of the fibril-monomer complex, and proton occupancy of each residue was analyzed by ^1H - ^{15}N HSQC spectrum, which has provided detailed information about the intermediate structure.

2P-084 Human p100 の SNase 様ドメインの折れ畳み能

Foldability of SNase-like domain in human p100.

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Staphylococcal nuclease (SNase) is a model protein for the study of protein folding and stability. Recently, a transcriptional regulator protein, p100, was found in human, which is composed of five SNase-like domains (SN domain). The crystal structure of the C-terminal domain of human p100 (residues 654-870, called TSN domain) showed that an additional domain called Tudor domain is inserted between the β_2 and β_7 of the SN domain. Although the sequence identity between SNase and the SN domain of TSN domain is only 15%, their tertiary structures are almost identical. In order to investigate whether or not the SN domain of p100 TSN domain by itself is an independent folding unit, the expression system were prepared and the TSN domain and the Tudor domain deleted SN domain can be obtained 20mg and 10mg from 400ml culture medium, respectively. Both the TSN domain and the SN domain take folded structures under a physiological condition, indicating that the SN domain is an independent folding unit, even if the internal Tudor domain was truncated. The results on the folding studies on the SN domain of p100 will be presented.

2P-085 ウマ β -ラクトグロブリンのフォールディングコアのペプチド解析

Peptide analyses of equine beta-lactoglobulin folding core

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Equine beta-lactoglobulin forms an expanded and helical conformation in the cold-denatured (C) state, which is a model of an early folding intermediate.

Recently, it was found that short peptides, G-peptide (97-110), H-peptide (110-128) and C-terminal peptide (124-138) were unstructured in isolation, while a longer fragment, CHIBL (88-142), showed helical structures similar to those in the C state. That is, long-range interactions are inevitable for helix formation in the C state and they reside in the CHIBL sequence. A possible long-range interaction is a disulfide bond (C106-C119). The helical contents of CHIBL are decreased by the disulfide reduction, indicating that the disulfide bond stabilizes helices. Proline scanning mutagenesis shows that the disulfide bond stabilizes not only helices including C106 and C119 but also the native-like C-terminal helix. To understand the mechanism by which the disulfide bond stabilizes the helices in CHIBL, we constructed GssH in which G- and H-peptides are linked by disulfide bond, a longer peptide HC (111-138), and a truncated CHIBL, CHIBL Δ F (97-142). Neither GssH nor HC can be structured, whereas CHIBL Δ F can form a stable helical conformation. These results indicate that the helices in CHIBL are stabilized by interhelical contacts and that the disulfide bond stabilizes them by increasing the effective concentration of helical segments.

2P-086 ジスルフィド結合を導入した高安定な低温ショック蛋白質変異体の分子間相互作用と熱力学特性

Molecular interaction and thermodynamic properties of highly stabilized cold shock protein mutant with a designed disulfide bond

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A mutant of cold shock protein from *Thermus thermophilus*, A11C/T40C, with a disulfide bond introduced on the molecular surface was designed based on the three-dimensional structure of the wild type, and the thermodynamic properties

and molecular function were evaluated by spectroscopic and calorimetric methods such as fluorescence and circular dichroism spectroscopy and differential scanning and isothermal titration calorimetry. The mutant was found to retain almost the same structure as that of the wild type in solution and show a monomeric and reversible 2-state-like thermal transition whose mid-point temperature was higher than that of the wild type by more than 10 K under the acidic pH condition. Although the main contribution for the stabilization was from the entropy factor, the observed entropy effect was far larger than expected one from the reduction of the chain entropy of the denatured state, and the enthalpy destabilization was also observed, indicating the thermodynamic properties of the native state were also affected by the mutation. The affinity to single-strand nucleic acids was evaluated by monitoring the thermodynamic interaction to single-strand oligo-DNAs with several kinds of length and sequence. The binding constant of the mutant was found to be lower than that of the wild type almost by 0.2-fold. The increase of the fluctuation of the native state of the mutant was strongly indicated by these results.

2P-087 リフォールディングモルチャリンの構造とダイナミクス

The conformation and dynamics of the refolded momorcharins

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The protein folding/unfolding process is reversible and it is generally known that the protein structure unfolded once could be refolded precisely again. But, some proteins give some different structures depending on the refolding conditions. In order to elucidate the protein folding mechanism, it may be the essential subject how precisely can the protein structure be refolded. Recently, we found the fluorescence resonance excitation energy transfer from tyrosine to tryptophan residue in the momorcharins and confirmed that it is a useful parameter to characterize the conformation state of the folding/unfolding intermediate. Two ribosomal inactivation proteins, alpha- and beta-momorcharin have similar amino acid sequences and tertiary structures each other. But they exhibited extremely different conformation and molecular dynamic properties at the folding/unfolding intermediate state formed by Gdn. When these momorcharins were equilibrated with 5 M of Gdn and then the denaturant was diluted to 0.5 M, the fluorescence maximum wavelengths both of two momorcharins shifted from 352 nm to 330 nm. This result suggested that the tertiary structures of these two momorcharins would be certainly refolded. But, the refolding of the local structure around the active site estimated by FRET and time-resolved fluorescence depolarization studies depended on the refolding condition and the characteristic of the folding/unfolding state of these proteins. The conformational and dynamical properties of the refolded momorcharins are discussed in detail.

2P-088 N-アセチルグルコサミン 3 量体結合によって、リゾチーム変異体 2SS (1+3)、2SS (3+4)、2SS (1+2) に誘導される秩序構造

Structures induced by tri-NAG binding to 2SS(1+3), 2SS(3+4) and 2SS(1+2) variants of lysozyme

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Two-disulfide variants of lysozyme lie on the boundary between non-native and native-like structures of lysozyme. We demonstrated that two-disulfide variants of 2SS(1+3) containing SS1 (C6-C127) and SS3 (C64-C80), and 2SS(3+4) containing SS3 and SS4 (Cys76-Cys94) lose the tertiary structure, but 2SS(1+2) containing SS1 and SS2 (C30-C115) has the native-like tertiary structure. So far, the effects of glycerol on inducing an ordered structure of 2SS-variants have been studied by us. This time, effects of tri-N-acetyl-D-glucosamine (triNAG) binding to 2SS-variants were examined. In the presence of 9 mM tri-NAG, 2SS(1+3) greatly recovered the tertiary structure, while 2SS(3+4) only slightly recovered the CD near at 293 nm even in the presence of 30 mM tri-NAG. In order to see the detail of the structure induced by tri-NAG binding, we carried out H/D exchange NMR experiments. It was contrary to our expectation that the increase in protection factors due to tri-NAG binding to 2SS(1+2) was only slight all over the residues. In addition, the heteronuclear relaxation rates were measured for individual residues of 2SS(1+2), and their dependence on internal motions was examined in order to see the details of unfolding of 2SS(1+2). Protection factors in 2SS(1+3) in water were equivalent to those in a random coil, but the increase in protection factors due to tri-NAG binding was surprisingly large almost everywhere. This means that not only the SS1 but also the SS3 is necessary for the construction of the binding site of tri-NAG.

2P-089 Adaptive multi-scale simulations of biomolecules for achieving high accuracy and high efficiency simultaneously

Adaptive multi-scale simulations of biomolecules for achieving high accuracy and high efficiency simultaneously

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Molecular dynamics (MD) simulation, as a complement of experimental methods, plays crucial roles in understanding the molecular mechanisms of many kinds of biological processes from molecular level due to its high temporal and spatial resolutions. For large protein systems, high accuracy atomistic MD simulation usually encounters sampling difficulty. In order to overcome this difficulty, some