- 2P092 β_2 -ミクログロブリンの酸変性と巻き戻り
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- 2aF09 Equilibrium and kinetics of β_2 -Microglobulin from the acid-unfolded state
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β_2 -Microglobulin (β_2 -m), 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. To gain the insight into the role of the intermediate in the folding and the amyloidogenesis of this protein, we investigated acid-induced unfolding of β_2 -m by monitoring far-UV circular dichroism (CD) and tryptophan fluorescence spectra and kinetic refolding from the acid-unfolded state and the intermediate state by stopped-flow fluorescence spectroscopy. Acid-induced unfolding of β_2 -m was completely reversible. The unfolding transition curve measured by far-UV CD showed that there were two transitions: (1) from the native state to the intermediate which is accumulated around pH 4.0; and (2) from the intermediate to acid-unfolded state. The results suggest that the intermediate exhibit the characteristics of the molten globule state. To elucidate the relationship between the equilibrium unfolding intermediate and the kinetic folding intermediate, we examined the kinetic refolding experiments with various combinations of initial and final pH values by using a stopped-flow technique. These results will be discussed.

- 2P094 非平衡条件下の蛋白質一分子の折りたたみ軌跡の直接観察
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- 2aF11 Direct observation of single-molecule trajectories of protein folding under non-equilibrium condition
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A protein is able to find its native structure among a myriad of unfolded structures much faster than the time scale expected for a random search. However, the mechanism of the efficient search remains unclear, because of limited experimental data on the direct observation of folding dynamics. Here, we developed a new single-molecule detection system that utilizes a combination of microscopic lens and sheath flow cell. The system enables us to detect single-molecule data with an improved S/N ratio, and furthermore to detect non-equilibrium folding trajectories after initiation of protein folding using rapid mixing.

We first applied this system to observing unfolded-state dynamics of iso1-cytochrome *c* labeled with Alexa 532 under mild-denaturing and equilibrium condition. At least 4 states were detected, and the transitions among these states were rare in the observation period of ~100 ms. The network analysis of the transitions showed the existence of a "hub" state, which is mildly collapsed and connects to all the other states.

We next detected folding trajectories of iso1-cytochrome *c* after a solution mixing to a physiological condition, in which the strong energy bias toward the native structure is present. The result demonstrated the appearance of at least two states, compact and extended states, after initiation of the folding and the rare transition between them. It suggests the presence of trapped states on the rugged energy landscape of folding even under a physiological condition.

- 2P093 水素/重水素交換 NMR 法によるヤギ α -ラクトアルブミンの天然及びモルテン・グロビュール状態の構造解析
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- 2aF10 The Native and Molten Globule States of Goat α -Lactalbumin Characterized by the NMR Hydrogen-exchange Technique
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Recombinant goat α -lactalbumin (GLA) is significantly less stable than the corresponding authentic one because the unfolding rate of the recombinant protein is faster than that of the authentic one. The destabilization is known to be caused by the presence of an extra methionine residue at the N-terminus in the recombinant protein. The stability of the δ E1 mutant GLA, which is genetically Glu1-deleted from the recombinant GLA, is known to be almost identical to that of the authentic GLA. In this study, we characterized structural details of the recombinant form, the authentic form and the δ E1 mutant of GLA in the native and the molten globule (MG) states by an NMR hydrogen-exchange technique. In the native state, significant protection (protection factor ~ 10^6 - 10^9) was observed in the C-helix and β -sheets involved within the β -domain in all the proteins. In the native state, the hydrogen-exchange rates of authentic GLA were remarkably slower than those of the recombinant one, reflecting differences in the stability between the two proteins. The authentic GLA and δ E1 mutant give similar protection against H/D exchange for all of the observable amide protons. In the MG state, significant protection (protection factor ~ 6-20) was observed in the C-helix. In the MG state, the hydrogen-exchange rates of authentic GLA are similar to those of the recombinant one. These results also indicate that the N-terminus of GLA is folded during the transition from the MG state to the native state.

- 2P095 ジスルフィド結合により安定化されているウマ β -ラクトグロブリンの中間体におけるヘリックス構造
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- 2aF12 A disulfide bond stabilizes the α -helices in the partially folded states of equine β -lactoglobulin
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Equine β -lactoglobulin (ELG) shows a variety of partially unfolded states. One of them is an expanded and helical conformation at low temperatures (C state). The secondary structures in the C state have been characterized through the proline-scanning mutagenesis experiment. It suggested that the regions corresponding to F, G, H strands and the major α -helix assume the native-like or nonnative helices. Peptides encompassing native-like and nonnative helices in the C state did not form stable helices. A longer fragment, CHIBL, which encompasses the structured region in the C states, showed a helical structure. CHIBL has a structure similar to the corresponding region of the full-length protein in the C state. This result indicates that non-local interactions responsible for helix formation in the C state reside in the sequence of CHIBL. The Cys106-Cys119 disulfide bond in CHIBL region links two nonnative helices in the C state. This is one of the possible long-range interactions stabilizing the helical structures. The CD spectra of disulfide-reduced ELG and disulfide-reduced CHIBL show decreased helical content. Therefore, helices both in ELG and in CHIBL are stabilized by this disulfide bond. The detailed structural analysis of the reduced ELG and reduced CHIBL by using proline mutants suggests that this disulfide bond contributes to the stabilities of not only the two helices linked by this disulfide bond but also other helices formed in the C state and CHIBL.