transition rate between energy minimum states. In the present study, we applied this method to a 28-residue all- β protein named the YJQ8 WW domain (PDB ID: 1EON). The simulations were performed under three different conditions: the main-chain ϕ and ψ dihedral angles of the residues that form the β -sheet in the native structure were restrained to the ranges of $\pm 60^\circ$, $\pm 90^\circ$, and $\pm 120^\circ$ centered on β -sheet's representative dihedral angles (ϕ , ψ) = $(-120^\circ, 130^\circ)$. With these secondary structure restraints, constant-temperature MD simulations were performed for 1 μ s at 400 K. We observed the folding into the native structure most frequently during the simulation with the restraints of $\pm 90^\circ$.

2P-096 Two transient intermediates appeared in the refolding process of Fyn SH3

Two transient intermediates appeared in the refolding process of Fyn SH3

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We have reported that an alpha-helix-rich intermediate appeared on the folding pathway of src SH3. This transient intermediate is rich in alpha-helix ($[\theta]_{222}$ is $-8,700 \text{ deg}^*\text{cm}^2\text{dmol}^{-1}$) and compact (Rg is 18.5 Å). We have also reported that, in contrast to src SH3, refolding of Fyn SH3 (W37F) at pH 6 was monophasic with the rate of 76 s^{-1} at 4C, but biphasic at -20C with the rates of 32 s^{-1} and 1.1 s^{-1} in the presence of 45% ethylene glycol. We have done the refolding of Fyn SH3 with another mutant (V11S, V15E, A39V, V55F). This mutant also showed biphasic with the rates of 19 s^{-1} and 1.4 s^{-1} at -28C in the presence of 45% ethylene glycol, whereas it showed nearly monophasic with the rate of 4.5 s^{-1} at -24C. The $[\theta]_{222}$ values of the burst, the amplitude of the fast phase and the amplitude of the slow phase at -28C were -8000, 7500 and 4400 in $\text{deg}^*\text{cm}^2\text{dmol}^{-1}$, whereas those of the burst and the amplitude of the kinetic phase at -24C were -5500 and 7300 in $\text{deg}^*\text{cm}^2\text{dmol}^{-1}$. Stopped-flow X-ray scattering experiments were also done. It shows monophasic at -28C. Rg values changed from 20.7 Å to 15.7 Å with the rate of 26 s^{-1} . These results can be summarized as follows; (1) Two mutants of Fyn SH3 (W37F and V11S/ V15E/ A39V/ V55F) showed two transient intermediates (TI1 and TI2) at subzero temperatures, though both of the intermediates are formed much more rapidly than in the case of src SH3. (2) TI1 is similar in Rg and CD amplitude of those of src SH3, whereas TI2 takes the same Rg with the native state, but the half CD amplitude.

2P-097 計算機による“理想的な”蛋白質のデノボ構造デザイン

De novo computational design of "ideal" protein structure

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Computational design of an arbitrary structure from scratch provides a stringent test of our knowledge of protein folding, and allows us to determine the essential factors that lead to foldable sequences. Kuhlman et al., succeeded in designing the novel protein structure of Top7 by using Rosetta. However, it was observed that Top7 folds differently from other similarly sized naturally occurring proteins: Top7 exhibits non-native intermediates upon folding. These intermediates may be present because Top7 was designed by optimizing its sequence to stabilize the native structure, rather than optimizing the sequence in the context of the global energy landscape. This optimization strategy is unlikely to work for designing larger and more complicated structures because non-native intermediates can induce aggregation and prevent proper folding. Here, we explored a rational method of de novo structure design of $\alpha+\beta$ proteins by explicitly designing sequences that have smooth funnel-like energy landscapes. Our results suggest that secondary structure (SS) length is an important parameter for de novo structure design. Optimal SS lengths eliminate non-native topologies by restricting the backbone structure, and producing smooth funnel-like energy landscapes. Moreover, we found a rule for selecting optimal SS lengths for creating $\alpha+\beta$ proteins. By using optimized SS lengths, we were able to design an ideal structure of a ferredoxin-like fold, which was experimentally validated using CD spectrum and NMR.

2P-098 新しいさや流セルを使用した蛋白質-分子の折り畳み軌跡の観測 Direct observation of single-molecule trajectories of protein folding using a new sheath flow cell

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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. Recently, we developed a technique for the detection of fluorescence signals from free single molecules for extended time periods. Here, we further developed the single-molecule system, in which a combination of microscopic lens and sheath flow cell was utilized. The improved system enables us to detect time-series data from single molecules more effectively. We applied this system to observe unfolded-state dynamics of iso1-cytochrome c labeled with a fluorescent dye under a mild-denaturing condition. Based on the intensity and dynamics of fluorescence traces from single molecules, we classified the traces into the 2 states, I and U. We further estimated the time scale of the effective equilibration time (EET) of the I state. EET was defined as the time that the cumulative intensity distribution of the time-series data from single molecules becomes

indistinguishable from that of the ensemble average. EET of the I state was estimated to be within 5 ms, suggesting that the conformational space of the I state is much smaller than the entire space. Thus, the formation of a folding intermediate might help to suppress the unnecessary conformational search during the folding.

2P-099 The transient and equilibrium intermediates of src SH3 refolding

The transient and equilibrium intermediates of src SH3 refolding

Xianju Jin, Jinsong Li, Masaji Shinjo, Yoshitaka Matsumura and Hiroshi Kihara (Dept of Physics, Kansai Medical University)

We have reported that an alpha-helix-rich transient intermediate (TI) appeared on src SH3 refolding pathway both at pH 3 and 6 at 4C (1). We have also reported that a mutant of src SH3 (A45G) formed an alpha-helix-rich equilibrium intermediate (EI) at pH3 (2). Rg values of TI were the same (20.2 ± 0.6 Å) regardless of pH and wt/A45G within experimental errors. In contrast, $[\theta]_{222}$ values are different at pHs 3 and 6 (wt type: $-8700 \text{ deg}^*\text{cm}^2\text{dmol}^{-1}$ at pH3 and $-6500 \text{ deg}^*\text{cm}^2\text{dmol}^{-1}$ at pH 6, A45G: $-6200 \text{ deg}^*\text{cm}^2\text{dmol}^{-1}$ at pH 6). Rg and $[\theta]_{222}$ value of EI of A45G at pH 3 were 19.1 Å and $-10300 \text{ deg}^*\text{cm}^2\text{dmol}^{-1}$, respectively. Thus, it is of interest to compare TI and EI. Recently, we have done refolding of A45G at pH 3 monitored by x-ray scattering, which showed no changes within a few seconds at 4C. This indicates EI and the TI of A45G at pH3 are the same in terms of Rg. We have also done pH-jump experiment of A45G from pH 3 to pH 6. It shows no burst phases. This suggests the conformational change of A45G from its EI to the native state at pH 6 does not pass through TI appeared at pH6 refolding process. These results suggest: (1) TIs at pH3 and pH 6 take the same Rg but different $[\theta]_{222}$ value. (2) TI at pH3 and EI are the same in Rg and $[\theta]_{222}$. (3) At pH-jump of A45G from pH 3 to 6 does not pass through TI (pH 6) on its isomerization pathway.1. Li et al. (2007) Biochemistry, 46, 5072-5082.2. Li et al. (2007) J. Mol. Biol., 372, 747-755.

2P-100 固体 NMR と TEM によるヒトカルシトニンの線維形成過程の測定と形成機構解析

Fibril formation process and reaction mechanism of hCT as studied by high-resolution solid-state NMR and transmission electron microscope

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Human calcitonin (hCT) is peptide thyroid hormone, plays a central role in calcium-phosphorus metabolism and has been realized close in relation to medullary carcinoma of the thyroid. hCT is known to form amyloid-like fibril in concentrated aqueous solution. We have previously showed that a π - π stacking between benzene rings of side chains in hCT can stabilize fibrils. We, therefore, examined the effect of particular phenyl ring using hCT mutants (F19L-hCT and F16L-hCT). This allows to determine the role of specific F in fibrillation by comparing the fibrillation rate of hCT mutant with that of Wt-hCT. ^{13}C NMR and TEM experiments were performed for the fibrillations at pH3.3 and 7.4. The ^{13}C NMR signals showed that structural transition of hCT mutants at pH3.3 occurred from random coil to β -sheet and random coil near Ala26 during fibrillation. The kinetic behavior of the fibrillation of hCT mutants was analyzed, by considering the two-step autocatalytic reaction mechanism, in which the first step is a fibril nucleation (k_1) and the second step is a fibril elongation (k_2). Rate constant, k_1 s of hCT mutants are determined to be as fast as those of Wt-hCT, while k_2 s of hCTs were decreased. These results suggest that the hCT mutants pronouncedly inhibit the fibril elongation process. Moreover, TEM experiments visually showed the initial step of fibrillation in hCT and the nuclei were clearly seen in the initial step. This observation supports that hCT fibril takes the two-step autocatalytic reaction mechanism.

2P-101 固体 NMR と TEM によるグルカゴンのバイセル膜存在下でのアミロイド様線維形成機構の解明

Fibrillation mechanism of glucagon in the presence of phospholipid bicelles as studied by ^{13}C solid-state NMR and TEM

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Glucagon is a 29 amino acid peptide hormone secreted by α -cells of pancreatic islet and plays a central role in the maintenance of normal circulating glucose. Glucagon is known to form amyloidogenic fibril which is cytotoxic through the activation of apoptotic signaling pathways. To understand fibril structures and fibrillation mechanism of glucagon under aqueous acetic acid solution (pH 3.3) in the presence of phospholipid bicelles as a mimic of living cell, ^{13}C solid-state NMR and TEM measurements were performed. In the process of glucagon fibril formation, α -helix changed to β -sheet structures around N-terminal and C-terminal regions in the absence of bicells as observed in ^{13}C NMR spectra. However, in the presence of bicells, glucagon fibril showed α -helix structures around N-terminal, whereas β -sheet structures around C-terminal. ^{13}C NMR signals of fibril grew after a certain delay time, suggesting that the fibrillation could be explained by a two step autocatalytic reaction mechanism. The first step is a homogeneous nuclear formation (rate constant of k_1), and the second step is a fibril elongation (rate constant of k_2). In the presence of bicells, k_1 became larger and k_2 became smaller than the case in the presence of bicells. From the measurement of TEM, it turned out that glucagon fibril strongly interacted with