multi-scale simulation methods have been proposed, in which atomistic MD Simulation is coupled to certain coarse grained (CG) MD simulation. However, the CG model is inherently less accurate and its simulation may deviate significantly from the average behavior of the atomistic potential. In this case, such coupling is not efficient and sometimes even bias the sampling of the atomistic simulation. In this work, a new multi-scale simulation protocol is proposed. In our method, we iteratively tune the CG potential according to the information of the atomistic potential and the sample conformations by the CG model. The test studies show that this new multi-scale simulation protocol is able to improve both the sampling of the atomistic simulation and the potential of the CG simulation within very limited steps of iterations. Our method is generic, and can be used to study any kinds of biomolecular systems, including protein folding, flexible protein-protein docking, and other conformational motions of biological importance.

2P-090 チオエステル基周辺の構造変化に伴ったエネルギーの変化に関する量子化学的研究

Energy profiles of thioester derivatives obtained by quantum chemical calculations

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Coenzyme A (coA) plays an important role in several enzymes. There are various derivatives of coA, in which various substituents bind to thiol group. acetyl-coA is one of the most important derivatives of coA, in which acetyl group binds to thiol of coA. Acetyl-coA is included in various enzymes, and it plays a significant role in human body. For the molecular mechanics study, which is one of computational methods for investigations of biopolymers, force field parameters of non-amino acid molecules such as co-factors should be defined. For acetyl-coA, parameters of thioester moiety were not determined, and the parameters were indispensable to molecular mechanics calculations of acetyl-coA. In this study, energy profiles of thioester were calculated by using quantum chemical calculations in order to determine the parameters. For the calculations, various methods and basis sets were used, and we investigated which methods were appropriate for thioester calculations. As the results, Hartree-Fock and MP2 methods gave more similar results to coupled-cluster methods, than density functional theory.

2P-091 蛍光法による酸性条件下での蛋白質変性中間体解析

The fluorescence spectroscopic characterization of the protein folding/unfolding intermediate state in the acidic condition

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The increase in the dipole moment by excitation of indole moiety of tryptophan residue experiences the energy relaxation under the strong dipole-dipole interactions resulting in large Stokes shift and has made the fluorescence of tryptophan residue a useful reporter for the structure, function and dynamics of protein. Recently, such dielectric relaxation process exhibited by the tryptophan residue is combined with the previous fluorescence decay analysis and quite new methods like the time resolved area normalized emission spectrum (TRANES) or time-resolved emission spectrum (TRES) are used for the studies on the interactions between the peptide elements and the conformation including the protein hydration. These methods enable the simultaneous analysis of both time- and energy-domain. Because of the higher spectroscopic sensibility and time-resolution power, the fluorescence spectroscopy is one of the powerful methods to analyze the subtle conformation change in the protein. In the present studies, the conformation and dynamics of molten globule state formed in the extremely acidic condition were investigated by the fluorescence spectroscopy such as TRANES, TRES and time-resolved depolarization. The characterization of the molten globule state is essential for elucidating the protein folding/unfolding mechanism, and the molten globule in the acidic condition is especially interesting because it is known to behave differently with others formed by the denaturants.

2P-092 分子動力学を用いた cucumber basic protein の構造に関する理論的研究

Theoretical study on structure of cucumber basic protein by means of molecular dynamics simulation

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Cucumber basic protein (CBP) is a kind of blue copper protein such as Plastocyanin and Azurin. These protein have a copper atom in their active site. It is well known that such a blue copper protein has a function of the electron transfer through the protein or membrane in vivo. The active site of CBP consists of one copper atom and four residues (His39, Cys79, His84, and Met89) which are similar to the active site structure of Plastocyanin. The charge transfer of the blue copper protein would be correlated with the oxidation and reduction of the active site. Especially the analysis of the electronic state and the structural stability of the active site in the oxidation and reduction condition would be important to reveal the mechanism of the electron transfer reaction. However, the detail of the charge transfer is still not clear due to the difficulty of experimental observation

of such process. In this study, at first, we estimate the electronic state and the interaction between atoms in the active site of oxidized type CBP (CBPox) and reduction type CBP (CBPre) by molecular orbital (MO) calculation. Next, we carried out the molecular dynamics (MD) simulation with estimated atomic charges and interaction parameters in the active site. From these calculations, we show the structural fluctuation and dynamics of the active site in these protein. The structural stability in solution is also discussed by estimating the solvation free energy of both CBPox and CBPre.

2P-093 網羅的アラニン挿入変異解析法によるジヒドロ葉酸還元酵素の機能発現及び構造形成領域の抽出

Extraction of the regions encoded foldability and/or functionality from dihydrofolate reductase by the systematic alanine insertion

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To design an artificial protein, it is essential to elucidate the building blocks of a foldable and functional polypeptide. We applied the systematic alanine insertion analysis to dihydrofolate reductase (DHFR) to extract the building blocks, called functional and structural elements, required for the functional expression and the structural formation. The DHFR activity of the alanine insertion mutants was screened by the TMP resistance assay, and the foldability of the mutants was examined by the solubility assay. In order to evaluate the functional and structural elements of DHFR quantitatively, we examined the structures and the enzymatic activities of the alanine insertion mutants by CD and spectroscopic measurement of NADPH. The alanine insertion mutants from 72nd-86th residue of DHFR, where the region from 74 to 81 is defined as a structural element, were purified. CD spectra indicated that the mutants from 74 to 81 take the non-native structure under a physiological condition, confirming that the region is the structural element. On the contrary, in several mutants from 78 to 83, the enzymatic activity was decreased by 30% due to the alanine insertions, despite that the TMP assay did not show any serious effect. The result suggests that the functional element determined by the TMP resistance assay only reflects the region required for the TMP binding, but neither the DHF reduction activity nor DHF binding. Further refinement on the functional element will be required.

2P-094 分子動力学を用いた溶液中にのAzurin(II)-シトクロム(II)複合体の構造安定性

Structural stability of Azurin(II)-Cytochrome(II) Complex in solution: A Molecular Dynamics Study

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Azurin (Az) exists in bacteria and is in a class of blue copper protein having copper atom in the active site. It is well known that Az forms complex in vivo by combining with cytochrome (Cyt) having hem iron in active site and the electron transfer occurs in these protein. This charge transfer would be attributed to the exchange of electron charge between active sites of each protein. However the details of charge transfer process are still not clear. In this study, we carried out three molecular dynamics (MD) simulations of the oxidized type Az and the reduction type Cyt and these complex and investigate the detail of complex structure and stability in solution. The active site of Az consists of one copper atom and five residues (Met121, His117, Cys112, Gly45, and His46) making coordination bond with copper atom. On the other hand, the active site of the cytochrome is constructed with Met61 and His16 residues binding to hem iron. In this study, at first, we estimate the electron state and the interactions between atoms in the active site of oxidation and reduction type of each protein, and then we carried out the MD simulation of a monomer and the complex with estimated interaction parameters. We also evaluate the solvation free energy of these monomer and complex in solution. From the difference of the calculated free energies, we mention the stability of the complex and affinity of Az and Cyt proteins.

2P-095 二次構造拘束つき分子動力学シミュレーションによる $\alpha 11-\beta$ タンパク質のフォールディングシミュレーション

Folding simulation of an $\alpha 11-\beta$ protein by molecular dynamics simulation with secondary structure restraints

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It takes longer time for a protein containing β -sheets to fold into its native structure because it has interactions between residues that are separated by a large distance in the primary sequence. Therefore, it has been difficult to observe folding of such proteins during molecular dynamics (MD) simulations. In general, the major obstacles for the folding simulation are too small probability of surmounting energy barriers in the ragged energy surface of a protein at physiological temperature and too large conformational space to be explored due to large internal degrees of freedom. To solve these problems, we have developed an MD method that uses secondary structure restraints to narrow down the conformational space to be sampled and uses a higher temperature to increase the

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 ringusing 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