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