studied the denatured structure of SNase in 0 to 4 M urea by measuring the lifetime of tryptophan triplet state (Trp-TS). The Trp-TS excited by nanosecond pulses is depopulated by the collision with cysteine (Cys). The lifetime of the Trp-TS is related to the distribution of the distance between Trp and Cys. For SNase Trp is located at the C-terminal but there is no Cys. We made four mutants that have Cys substituting for 13th, 44th, 82nd, and 120th threonine. The Trp-TS was depopulated with the lifetime of about 30 μ s for all the mutants in the absence of urea. The lifetime of the mutants is the same as that of the wild type because the quenching by Cys is negligible in the native SNase, where Cys is separated from Trp by more than 1 nm. The difference in the lifetime between the mutants is expected in the denatured SNase if there is the residual structure.

1P018 ラマン円偏光二色性分光法の開発と生体関連分子への応用 Exploring the Protein Structures by Raman Optical Activity

Masashi Unno, Takahito Shingae, (Department of Chemistry and Applied Chemistry, Saga University)

Understanding protein function at the atomic level is a major challenge in a field of biophysics and requires the combined efforts of structural and functional methods. Raman spectroscopy has been widely used for such studies and provides valuable information concerning the active site as well as protein structures. It is, however, desired to develop a new method that allows us to investigate molecular structures in further details. A potential technique for structural elucidations of biological molecules is Raman optical activity (ROA), which is a spectroscopic method with a high sensitivity to the structural details of chiral molecules. Although this method has been proven to be useful for structural studies on biological samples, its application to biological samples is limited to non-fluorescent samples. Thus we have constructed ROA spectrometer using near-infrared (NIR) laser excitation at 785 nm. The NIR excitation enables us to measure ROA spectra for a variety of biological samples, including photoreceptor proteins, without fluorescence from the samples.

1P019 セレン含有アミノ酸の立体構造に関する量子化学的研究 A computational study for structural features of selenium-containing amino acids using quantum chemical calculations

Akifumi Oda^{1,2}, Ohgi Takahashi¹, Yu Takano², (¹ Faculty of Pharm Sci, Tohoku Pharm Univ.; ² Institute for Protein Research, Osaka Univ)

For the classical molecular mechanics calculations, although force field parameters for period 2 elements were established and are widely used, the development of parameters for period 3 and 4 elements have not been enough carried out. We previously developed force field parameters of sulfur in thioesters. However, for period 4 nonmetal elements, such as selenium, only few studies were carried out. On the other hand, there are amino acids including selenium in proteins. The selenocysteine plays an important role in some enzymes, and the selenomethionine is used for X-ray diffraction studies of proteins. Thus, computational chemical studies of selenium-containing amino acids are indispensable to investigations of the proteins including selenium. In this study, quantum chemical calculations of the amino acids including selenium were carried out. For the calculations, density functional theory (DFT) and ab initio molecular orbital methods were used. Several types of basis sets were used, such as Pople-type basis sets and effective core potential. For the model molecule, structural optimizations were carried out by using several model chemistries, and the structural features were compared. These results can be applied for structural bioinformatics studies and drug design trials for selenium-containing proteins.

1P020 H-ras GTP 複合体の溶液中の立体構造に関する分子動力学シミュレーション Molecular dynamics simulation of the structures of H-ras GTP complexes in solution

Takeshi Miyakawa¹, Ryota Morikawa¹, Masako Takasu¹, Hidemi Nagao², (¹ Tokyo University of Pharmacy and Life Sciences; ² Kanazawa University)

H-ras proteins, the products of the *ras* onco- and protooncogenes, are guanine binding proteins, which act as molecular switch. In the active state they are bound to guanosine triphosphate (GTP), and in order to switch to the inactive state, the gamma-phosphate of the nucleotide has to be hydrolyzed. In the oncogenic mutation, this reaction is suppressed. Understanding of this reaction is very important to overcome human cancer.

Molecular dynamics simulations of the structures of H-ras GTP complexes in solution have been performed based on the force field and charges for GTP and Mg²⁺ created in the Hartree-Fock level, using a basis functional set, 6-31G** [1].

Recently free energy calculations of docking structure of complex systems were performed[2].

We recreated the force field and charges for GTP and Mg²⁺ in the H-ras using Gaussian 09 [3]. Calculations were performed in the B3LYP level, which gives more information than Hartree-Fock level, using a basis functional set 6-31G**.

We will evaluate the physical quantities, such as the binding energy of GTP, of the solution structures of H-ras GTP complexes, by applying the potential developed above and performing the molecular dynamics simulation of the solution structures of H-ras GTP complexes.

1P021 Conformational transitions in the CDRH3 region of the anti-dansyl monoclonal antibody by molecular dynamics simulations

Narutoshi Kamiya¹, Jinzen Ikebe², Koji Umezawa², Yasushige Yonezawa¹, Junichi Higo¹, Haruki Nakamura¹, (¹ Institute for Protein Research, Osaka University; ² Grad. Sch. of Frontier Biosciences, Osaka University)

Molecular recognition plays an important role in biological systems such as between antibody and antigen in the immune system. Induced-fit model is known to involve conformation changes of protein in forming the final ligand binding complex. Recently, population shift model postulates that all protein conformations pre-exist in the conformational ensemble, and the ligand selects the most favored one. Conformational changes occur in the CDRH3 loop region of anti-dansyl monoclonal antibody upon binding of a small ligand antigen (i.e., dansyl-lysine). In this study, we execute molecular dynamics (MD) simulation to clarify the molecular recognition mechanism of anti-dansyl monoclonal antibody.

Both apo- and holo-structures of anti-dansyl monoclonal antibody fragment have been reported. We executed constant NPT MD simulations, where N is number of particles; P is pressure (= 1 bar); T is temperature (= 300 K), starting from apo- or holo-conformation of the antibody. Here, ligand molecule was not included.

Conformational transitions between apo- and holo-conformations in the CDRH3 region were found in both simulations. We characterized the transitions by one-dimensional reaction coordinate and analyzed potential of mean force along the reaction coordinate. The energy barriers between apo- and holo-conformations were less than a few kT. Therefore, apo- and holo-conformations pre-exist in the ligand free form of anti-dansyl monoclonal antibody, and the molecular recognition of the antibody is explained by the population-shift model.

1P022 トランスフェリンとトランスフェリン受容体2との複合体形成における結合サイトの予測 Prediction of Contact Sites between Transferrin and Transferrin Receptor2

Tetsuya Sakajiri¹, Hirofumi Yajima², Takeshi Kikuchi³, Motoyuki Shimonaka², Takaki Yamamura¹, (¹ The University of Morioka; ² Tokyo University of Science; ³ Ritsumeikan University)

The main role of Transferrin receptor2 (TfR2) is uptake of transferrin (Tf)-bound iron into the cell as well as that of TfR1. Diferric Tf (Fe₂Tf) is bound to TfR (TfR1 or TfR2) at the cell surface (pH 7.4), followed by endocytosis and iron release due to acidification (pH 5.6) in the endosome. The apoTf remains bound to the TfR in the endosome. The apoTf-TfR returns to the cell surface and the apoTf is dissociated from the TfR, allowing it to be recycled. TfR2 also has an Arg-Gly-Asp (RGD) motif which is crucially involved in the formation of Fe₂Tf-TfR1 or apoTf-TfR1. However, TfR2 and Fe₂/apoTf-TfR2 structures have not yet been determined. In this study, in order to predict contact sites between Tf and TfR2, we attempted to build the structure of TfR2 using a homology modeling technique based on the TfR1 crystal structure. Then, we created structures of Fe₂/apoTf-TfR2 complexes on the basis of the structures of Fe₂/apoTf-TfR1 complexes modeled in our previous study (Protein J, v.28, pp.407-414, 2009). Consequently, we additionally found two specific ionic bonds in the Fe₂Tf-TfR2 complex, whereas did disappearance of three ionic bonds due to replacement of charged residues in TfR1 with neutral residues. The decrease in one ionic bond probably results in 30-times lower affinity of Fe₂Tf to TfR2 than to TfR1. The apoTf-TfR2 complex had other two ionic bonds alternative to two ionic bonds occurring in the apoTf-TfR1 complex.

1P023 キャピティ体積計算による疎水性変異蛋白質の熱安定性の予測 Prediction of the thermal stability of hydrophobic mutants by cavity volume calculations

Masahiro Kato, Jun Nakajima, Kousuke Sasaya, Eri Asari, Takeshi Kousaka, Minoru Saito, (The Graduate School of Science and Technology, Hiroshima University)

Cavities in proteins have been interesting for protein scientists in connection with the thermal stabilities. To investigate the relationship between the stability and the cavity, mutant proteins with cavities were created by substituting