of ubiquitin) at low and high pressure values and compared them. We found that the potential energy and volume of the system were widely distributed in the simulations as the results of a random walk in pressure space. Moreover, we calculated the probability distributions of dihedral angles and principal components at low and high pressure values. The PTST simulations were successfully performed. We also found that the water molecules near the protein played an important role in the conformational change of ubiquitin at high pressure.

1H1348 内部運動のゲートウェイとなるキャビティ水和: T4 リゾチームの高圧¹³C-, ¹⁵N- NMR 研究

Cavity hydration as a gateway to internal mobility: A high pressure ¹³C and ¹⁵N NMR study of T4 lysozyme

Akihiro Maeno¹, Ryo Kitahara², Frederick Dahlquist³, Shigeyuki Yokoyama⁴, Frans Mulder⁵, Kazuyuki Akasaka⁶ (¹*Grad. Sch. of Biology-Oriented Sci. and Tec., Kinki Univ.*, ²*Pharm. Sci., Ritsumeikan Univ.*, ³*Inst. Mol. Biol. and Dept. Chem., Univ. of Oregon*, ⁴*Grad. Sch. of Sci., Univ. of Tokyo*, ⁵*Dept. Biophys. Chem., Univ. of Groningen*, ⁶*High Pressure Protein Research Center, Kinki Univ.*)

Conformational heterogeneity and fluctuations are key to function in globular proteins. Here we investigate the role of internal cavity and its hydration on the conformational heterogeneity and fluctuations of a globular protein using a cavity mutant of T4 lysozyme. We use pressure (3 MPa - 300 MPa) as a means to cause hydration of the protein interior and observe the conformational heterogeneity and mobility of the side chain methyl groups by ¹³C/¹H and the backbone amide groups by ¹⁵N/¹H two-dimensional NMR. Below 100 MPa, the ¹³C/¹H NMR signals from the side-chain methyl groups that surround the largest cavity are preferentially reduced and disappeared. Above 150MPa, some methyl peaks around small cavities far from large cavity showed a preferential reduction of peak intensity, while other methyl peaks were gradually broadened. The result suggests that the hydration and local disorder of conformation which initially at the large cavity now propagates into the periphery regions around other cavities. We also found the similar trend of line-broadening for the backbone amide groups on the way to pressure-induced total unfolding, suggesting that the internal cavity hydration is the primary event within the folded ensemble and that the hydration propagates in the protein matrix to further distance to increase the heterogeneity and mobility within the folded manifold.

1H1412 変性の入り口としてのギャビティの水和: 高圧 NMR によるリゾチームの低温変性過程の観測

Cavity hydration as a gateway to unfolding: An NMR study of hen lysozyme at high pressure and low temperature

Yuji O. Kamatari¹, Kazuyuki Akasaka² (¹*Life Science Research Center, Gifu University*, ²*Institute of Advanced Technology, Kinki University*)

We have used low temperatures (down to -20°C) and high pressures (up to 2000 bar) to populate low-lying excited state conformers of hen lysozyme, and have analyzed their structures site-specifically using ¹⁵N/¹H two-dimensional HSQC NMR spectroscopy. The resonances of a number of residues were found to be selectively broadened, as the temperature was lowered at a pressure of 2000 bar. The resulting disappearance of cross-peaks includes those of residues in the β-domain of the protein and the cleft between the β- and α-domains, both located close to water-containing cavities. The results indicate that low-lying excited state conformers of hen lysozyme are characterized by slowly fluctuating local conformations around these cavities, attributed to the opportunities for water molecules to penetrate into the cavities¹. Furthermore, we have found that these water-containing cavities are conserved in similar positions in lysozymes from a range of different biological species, indicating that they are a common evolutionary feature of this family of enzymes¹.

References :

1. Y. O. Kamatari, L. J. Smith, C. M. Dobson, and K. Akasaka, (2011), *Biophys. Chem.*, 156, 24-30, (2011).

1H1424 小さな球状蛋白質の部分モル体積への温度変化の影響

Temperature Effects on Partial Molar Volume of a Small Globular Protein by using Extended Scaled Particle Theory

Hiroto Tsuji, Masayuki Iriza (*Bioscience and Bioinformatics, Systems Engineering and Computer Science, Kyushu Institute of Technology*)

We have calculated partial molar volumes of a small globular protein, bovine

pancreatic trypsin inhibitor (BPTI), in water by using extended scaled particle theory, which has been developed by us for an arbitrary shaped solute molecule. The calculated volumes have a minimum at a temperature of 23 Celsius degree. Our method quantitatively reproduces hydrophobic hydration effects on the partial molar volumes with analytical expressions. The main contribution from hydrophobic hydration is found to be bilinear of two factors; the one is the pressure dependence of bulk water density, the other is the structure of the protein. In the case of smaller hydrophobic molecules (alkanes or alcohols), calculated partial molar volumes also have a minimum at the same temperature. Recent experimental works on the partial molar volumes of an alcohol have revealed the minimum by adding the experimental values at low temperatures, where the data were insufficient. Preceding experiments of the partial molar volumes of an amino acid showed the contribution from its hydrophobic side chain also had the minimum at the same temperature. But the whole values of partial molar volume of the amino acid were strongly affected by the charges on N and C terminals and the minimum disappeared. Our results suggest separating the hydrophobic and hydrophilic contributions for understanding the temperature effects on the partial molar volumes.

1H1436 蛋白質-蛋白質複合体のホットスポットにおける水のエントロピー効果の重要性

Crucial Importance of Water-Entropy Effect for Hot Spots in Protein-Protein Complexes

Hiraku Oshima¹, Satoshi Yasuda², Takashi Yoshidome¹, Mitsunori Ikeguchi³, Masahiro Kinoshita¹ (¹*Institute of Advanced Energy, Kyoto University*, ²*Graduate School of Energy Science, Kyoto University*, ³*Graduate School of Nanobioscience, Yokohama City University*)

“Hot spots” are residues accounting for the majority of the protein-protein binding free energy (BFE) despite that they comprise only a small fraction of the protein-protein interface. A hot spot can be found experimentally by measuring the BFE change upon mutating it to alanine: The mutation gives rise to a significantly large increase in the BFE. Theoretical prediction of hot spots is an enthusiastic subject in biophysics, biochemistry, and bioinformatics. For the development of a reliable prediction method, it is essential to understand the physical origin of hot spots. To this end, we calculate the water-entropy gains upon the binding both for a wild-type complex and for its mutant complex using a hybrid method of the angle-dependent integral equation theory applied to a molecular model for water and the morphometric approach. The BFE change due to alanine mutation is evaluated only from the change in the water-entropy gain with no parameters fitted to the experimental data. It is shown that the overall performance of predicting hot spots in our method is higher than that in Robetta, a standard free-energy-based method using fitting parameters, when the most widely used criterion for defining an actual hot spot is adopted. This result strongly suggests that the water-entropy effect we calculate is the key factor governing basic physics of hot spots.

1H1448 高圧 NMR 法で決定された高エネルギー状態が安定化されたユビキチン変異体の合理的設計と物性

A design strategy of high-energy states of proteins based on high pressure NMR study

Souichiro Kitazawa¹, Maho Yagi^{2,3}, Kenji Sugase⁴, Koichi Kato^{2,3}, Ryo Kitahara¹ (¹*Ritsumeikan Univ.*, ²*Okazaki Institute for Integrative Bioscience*, ³*Nagoya City Univ.*, ⁴*Suntory Foundation for Life Sciences*)

By using high-pressure NMR spectroscopy, we found that ubiquitin is considered to exist as an equilibrium mixture of the basic folded conformer N₁, alternatively folded conformer N₂, locally disordered conformer I and totally unfolded conformer U. Interestingly, similar conformational equilibria were conserved only among ubiquitin and its homologues NEDD8 and SUMO2. Here, we demonstrate a rational design for N₂ by a single amino acid replacement method based on the solution structure of the ubiquitin at 3 kbar previously reported by high pressure NMR spectroscopy (Kitahara et al JMB 2005) and structural alignment of ubiquitin fold proteins (Kiel and Serano JMB 2006). According to the N₂ structure of WT ubiquitin at 3 kbar, substantial conformational changes are observed at the C-terminal region of the protein including the α-helix and C-terminal strand. There are two important interactions: one is a hydrogen bond between I36 backbone carbonyl group and Q41 side-chain amide group, which is conserved widely in ubiquitin family. The other is a salt-bridge between K11 amino group and the E34 carboxyl group. We think that the two interactions are key factors for controlling the N₁-N₂ conformational fluctuation of ubiquitin. The culmination of multiple NMR