accumulation of GFP, alkaline phosphatase (PAP), and diluted PURE system with active forms. Especially, accumulation rate of PAP reached 25-fold. The methods also succeeded to accumulate DDW-S30. The accumulated DDW-S30 showed 180 mg/ml protein concentration, which expected to contain ca. 260 mg/ml of macromolecules, and showed equivalent activity of cell-free protein production to the DDW-S30 before accumulation. Such an accumulated DDW-S30 has a potential to be a material for revealing the difference between cells and materials, and is expected to contribute synthesizing cells from cell extract in future.

1E1510 TMAO による分子クラウディング環境への蛋白質移相自由エネルギーの三次元描像

Three-Dimensional Imaging of the Protein Transfer Free Energy into the Molecular Crowding Condition with TMAO

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Some of the living organisms in the extreme environments contain various organic cosolvents (osmolytes) in their cells. These osmolytes protect the conformation and the function of biomolecules from environmental stresses, such as high temperature or high pressure.

In this study, cytoplasmic conditions of the extremophiles were mimicked by aqueous solution of 2.2M TMAO (trimethylamine N-oxide: osmolyte found in deep sea fishes). The volume fraction of the TMAO as crowder was set to mimic the typical molecular crowding condition in the cytoplasm. Under such conditions, transfer free energy (TFE: the change in Gibbs energy of the solute when it transfers from the solvent phase into a different phase) of apomyoglobin were calculated by Kirkwood-Buff (KB) theory using the atomic configurations of the solvent molecules generated by MD simulations. The simulated TFE and the preferential interaction parameter correlated favorably with experimental values. Furthermore, 3-dimensional distribution of the TFE were revealed with our developed space-time deconvolution techniques of the KB integrals. The results indicated that the positive TFE regions have planer shapes, and are continuously distributed along the surface of protein. In contrast, the negative TFE regions have spherical shapes and are scattered mainly around the side-chains [1]. The correlation between TFE distribution and the chemical or geometric property of the protein surface will be discussed.

[1] Yu, I; Nakada, K.; Nagaoka, M., J. Phys. Chem. B, 2012, 116, 4080-4088.

1E1522 シトクロム *c* に対する尿素効果の自由エネルギー解析

Free-energy analysis of urea effect on cytochrome *c*

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Urea is a widely used denaturant for protein. The mechanism of the denaturation has not been clearly determined though its effect has been discussed in both experimental and theoretical studies. At the moment, the driving force of denaturation is explained by the following two mechanisms. One is the direct mechanism. In the direct mechanism, the denaturation is caused by the direct interaction between protein and urea. Urea is considered to interact with hydrophilic residues and backbone via the electrostatic interactions, and with large, heavy residues via the van der Waals interactions. The other is the indirect mechanism. In the indirect mechanism, urea disturbs the water structure such as hydrogen bonding, and the hydrophobic core is exposed to the solvent. The calculation of salvation free energy may provide a key for elucidating the urea effect if the contributions of water and urea are obtained individually. In this study, we calculated the solvation free energies of cytochrome *c* immersed in pure water and that in urea solution by using the molecular dynamics simulation coupled with the method of energy representation. The transfer free energy is always negative, and the transfer free energy from pure water to urea solution is found to be strongly correlated to the van der Waals interaction between solute and solvent molecules. The result supports the “direct” mechanism and the dominance of the van der Waals interaction in the urea effect on protein.

1E1534 タンパク質の水和水の水素結合ダイナミクスとタンパク質ダイナミクスとの動的カップリング

Kinetics of hydrogen-bonding of protein hydration water and its dynamical coupling with protein dynamics

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Unique physical properties of water are originated from the hydrogen bond. The hydrogen bond determines the spatial network patterns and water dynamics. Hydration water around the molecules, such as ions, polymers and bio-molecules, has quite different physical properties from bulk water. The protein hydration affects the biological function of the protein. Here, by using the molecular dynamics simulation, we show that the hydrogen bond kinetics of the protein hydration water is strongly affected by the local bonding patterns near the tagged hydrogen bond and that percolation of hydration water on the protein surface essentially determines the cooperative hydrogen bond forming and breaking dynamics. These dynamical characters of hydration water are quite different from those of the bulk water. The hydrogen bond kinetics between hydration water is correlated with the kinetics between the protein and hydration water, suggesting the dynamical coupling between them. The percolation of hydrogen bond network would contribute to the physical properties of protein hydration water. This hydration water dynamics induces the protein dynamical transition observed by neutron inelastic scattering. The dynamical coupling between hydration water and protein should be essential for protein thermal fluctuation and functionality.

1E1546 テラヘルツ時間領域分光を用いた天然および変性状態タンパク質における低振動スペクトルの水および温度依存性の観測

Temperature and hydration effects of low-frequency spectra of native and denatured proteins studied by terahertz time-domain spectroscopy

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Theoretical calculations have shown that in the pico-second region proteins exhibit collective vibrational motions. These modes can be referred as low-frequency modes, since these modes usually appear in several wavenumber regions, or terahertz frequency regions. It is known that these motions are usually well correlated with large conformational changes that are necessary for protein functional expression. Until now a lot of studies focusing on hydration and temperature dependences on protein dynamics have been done by using a large number of spectroscopic methods since these parameters are deeply connected to the protein function. Though it has been proved that “dynamical transition” is universally observed at around 200 K, little is known about the effect of hydration. In this study we measured low-frequency dynamics of native and chemically denatured lysozyme to understand the effect of hydration by using terahertz time-domain spectroscopy. Difference spectra between dried and hydrated states indicate that above the dynamical transition temperature the low-frequency dynamics were activated. In contrast, the differential spectra below the dynamical transition point show complicated features. Below 25 cm⁻¹ absorption coefficients of the difference spectra are negative, and above this wavenumber the values increase as the wavenumber increasing. We discuss about the interpretation of these observations and differences between the native and denatured states.

1E1558 誘電緩和分光法によるアルキルカルボン酸の水和特性

Hydration Properties of Alkyl Carboxylate Series by Dielectric Relaxation Spectroscopy

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We measured hydration properties of sodium carboxylate series A: C_nH_{2n+1}COONa (n=1-5), and B: C₄H_{9-m}(COONa)_m (m=2-4) in a low concentration range (<0.09 M) at 20.0 ± 0.01 °C by dielectric relaxation (DR) spectroscopy in the frequency range 0.2-26 GHz. The DR spectra were decomposed into several Debye components and the bulk water component by Hanai mixture theory. Their DR parameters were determined by least fitting. As results, a Debye component with a DR frequency (f_c) of ~18 GHz, which is higher than bulk frequency (17.0 GHz at 20 °C) hence assigned as hyper-mobile water (HMW), and another component with lower f_c (5.3-8.5 GHz) assigned as constrained water (CW) were found around solutes of series B, while only the 5.3-8.5 GHz component (CW) was found around series A. An error propagation analysis assured the resolution of f_c ~0.5 GHz, supporting the existence of these components distinguished from the bulk water component. By fixed volume analysis, we found that: 1) with increasing the length of hydrophobic alkyl group, CW increased while bulk water decreased; 2) with increasing the number of carboxylic group, HMW increased and bulk water decreased. Also, both HMW and CW were found around HCOONa (0.06M) at 10.0 ± 0.01 °C. In