approximately divided into 11 independent relaxational modes, about half or five of which with larger amplitudes contribute to 76% of the whole fluctuation. (3) Each of these major modes originates in correlated fluctuations of the hydration states of several specific PGs. Their relaxation times range widely from 10 ps to several tens of ns.

1PT010 誘電緩和分光測定によるオリゴリン酸塩の水和解析

Hydration study of oligophosphates by dielectric relaxation spectroscopy

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Adenosine triphosphate (ATP) is an energy mediator in biological systems. Recently, the importance of the hydration effects on ATP hydrolysis free energy is being realized widely. In this study, we performed high-resolution dielectric relaxation spectroscopy (DRS) for oligophosphates aqueous solution (orthophosphate, pyrophosphate and triphosphate; $\leq 20\text{mM}$) as a model of adenosine oligophosphates. The present DRS analysis can divide dielectric properties of the solution into multiple relaxation components. Experiments were performed by microwave network analyzer on 0.2-26 GHz frequency range with the sample solution controlled at $20.0 \pm 0.01^\circ\text{C}$.

Firstly, we found two types of water that have different relaxation frequency (5 GHz, 20 GHz) from bulk water (17 GHz) around solute ions. The 5-GHz component was attributed to the constrained water, and the 20-GHz component to the hyper-mobile water; HMW.

Secondly, with increasing valence of solute ions, the dispersion intensity of HMW increased significantly. It suggested that the hydration state of oligophosphates was strongly influenced by the valence rather than the polymerized structure of phosphate unit.

1PT011 ATP, ADP, Pi の水溶液から脂肪酸アミン / オクタノール溶媒への移行：その熱力学特性とメカニズム

Thermodynamics and mechanism of transfer of ATP, ADP and Pi from aqueous solution to fatty acid amine/octanol solvent

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ATP hydrolysis is an important exergonic reaction driving the thermodynamically unfavorable reactions in living systems. Although hydration is considered to contribute significantly to energy profiles of chemical reactions in aqueous condition, the extent of its contribution to ATP hydrolysis remains to be experimentally assessed. In order to evaluate the effect of hydration on the thermodynamics of ATP hydrolysis, ATP hydrolysis in non aqueous condition (i.e., organic solvent) has been studied. For this purpose, fatty acid amine-containing octanol was used as a model solvent, in which ATP, ADP and Pi are highly dissolved. Approximate hydration energies were estimated from the partition coefficients as Gibbs energy changes (ΔG) for transfer of ATP, ADP and Pi from aqueous solution to the fatty acid amine-containing octanol, and compared to ΔG for hydrolysis in the solvent. ΔG for the transfer was smaller than that for hydrolysis in the solvent. Thus, the hydration energy may modestly modulate the Gibbs energy of ATP hydrolysis in aqueous solution.

This energy profile is going to be refined by consideration of the enthalpy changes for the transfers, which can be estimated from van't Hoff plots of temperature-dependencies of the partition coefficients. In addition, in order to elucidate how ATP, ADP and Pi are stabilized in the solvent, ionizations of ATP, ADP and Pi and reversed micelle formation will be investigated by pH titration and dynamic light scattering, respectively.

1PT101 リガンド結合状態及び非結合状態のケナガマンモスヘモグロビンの結晶構造

The crystal structures of hemoglobin from the woolly mammoth in liganded and unliganded states

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We have recreated the hemoglobin (Hb) of the extinct woolly mammoth using artificial genes expressed in *Escherichia coli*. The globin sequences were previously determined using DNA recovered from frozen cadavers. Although highly similar to the Hb of existing elephants, the woolly mammoth protein

shows rather different responses to chloride ions and temperature. In particular, the heat of oxygenation is found to be much lower in the mammoth than in the elephant protein, which appears to be an adaptation to the harsh Siberian winter. Many Arctic animals such as reindeer show a similarly low temperature dependence of oxygen affinity in order to ensure oxygen transport to the limbs, which may experience much lower temperatures than the body core, even in large, active and fur-covered mammals. In order to discover the structural basis for the altered heterotropic effect we have determined the crystal structures of the Hb in the deoxy, carbonmonoxy and aquo-met forms. These models, the first protein structures from any extinct species, show many features reminiscent of human Hb, but underline how delicate control of oxygen affinity relies on much more than overall quaternary structure changes.

1PT102 HIV-1 プロテアーゼによるダルナビル耐性の分子機構の解明

Structural change at the flap region of HIV-1 protease associated with darunavir resistance

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Darunavir (DRV) is the most potent protease (PR) inhibitor (PI) currently available for anti-HIV therapy. It has a higher genetic barrier to resistance development than the other PIs. However, it has been reported that DRV is vulnerable to the resistance acquisition, especially for the patients who have previously failed the PI-containing treatments. Here, in order to understand the basis of DRV-resistance, we analyzed structures of the DRV-resistant PR.

We first induced DRV-resistant virus in the culture system with the DRV concentration increased over 154 days. On day 154, we isolated the DRV-resistant virus with 55.5 times higher IC50 against DRV than the wild-type (WT) clone. The analysis of the viral sequences revealed that the escape mutant with five mutations in PR was dominantly induced during the viral replications. Next, we determined a high-resolution (1.8 Å) crystal structure of the DRV-resistant PR. It turns out that the DRV-resistant PR has an open configuration at its flap region, which has not been observed in WT PR. Furthermore, the motional properties and DRV-binding modes of the PR were analyzed by molecular dynamics (MD) simulations. MD simulations showed that the flap region of DRV-resistant PR is more flexible than WT, and that the DRV-interaction modes are distinct. The results suggest that structural change at the flap region is involved in the resistance development of HIV-1 PR. Our findings will provide useful information to develop effective PIs even to the DRV-resistant HIV-1.

1PT103 P2X 受容体の ATP 認識機構およびチャネル活性化機構

1YS1030 Mechanism of ATP binding and channel activation in P2X receptors

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ATP is known as the vital energy source involved in energy metabolism, biosynthetic reactions and active transport. It is also an essential extracellular signaling molecule that activates two distinct families of ATP receptors: ionotropic P2X and G-protein-coupled P2Y receptors.

P2X receptors are trimeric ATP-activated ion channels permeable to Na^+ , K^+ and Ca^{2+} , expressed throughout the human body including the nervous, cardiovascular and immune systems and are implicated in various physiological processes such as synaptic transmission, smooth muscle contraction, taste and pain sensing and inflammation.

A recent crystal structure of zebrafish P2X4 receptor in an apo, closed state, revealed the chalice-shaped trimeric architecture of P2X receptors. However, due to the lack of a crystal structure in complex with ATP, the ATP binding site and the mechanism of channel activation remained unclear.

Here we report the crystal structures of zebrafish P2X4 receptor in the presence and absence of ATP at 2.8 and 2.9 Å resolution, respectively. The agonist-bound structure reveals a novel ATP binding motif and an open channel pore conformation. A structural comparison of two states suggests that ATP binding induces cleft closure in an intersubunit agonist binding site, which expands the region of the receptor proximal to the transmembrane domain, causing an iris-like opening of the channel pore. This work provides the first structural insights into how ATP binding activates P2X receptors based on atomic resolution structures.