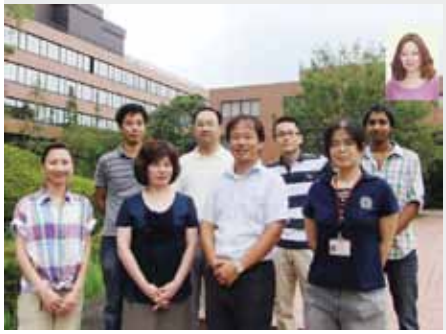
Investigation of Molecular Mechanisms of Transporters and Receptors in Membrane

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Sensing



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Membrane proteins are important for homeostasis of living cells, which work as ion channel, ion pump, various types of chemical and biophysical sensors, and so on. These proteins are considered as important targets for biophysical studies. However, their molecular mechanisms have not been studied well, because X-ray crystallography and NMR spectroscopy are hard to access them in general.

Our main goal is to clarify molecular mechanisms of transporters and receptors in cell membrane mainly by using stimulus-induced difference infrared (IR) spectroscopy, which is sensitive to the structural and environmental changes of organic and bio- molecules. In this year, Dr. Kimura has started to construct a microfluidic device to monitor biological and chemical reactions by infrared and fluorescent microscopic techniques.

1. Perfusion-Induced Difference FT-IR Spectroscopy for Na^+-Pump V-ATPase

Attenuated total reflection (ATR) FT-IR spectroscopy is a powerful technique to obtain infrared spectra of membrane proteins immersed in aqueous solution.[1,2] By exchanging buffer with and without salts, the difference spectra between the two conditions provide the structural information relating to the interaction between protein and ions. In this year, application of this technique to a transporter protein (V-ATPase) has been published in JACS.

V-ATPase from *Enterococcus hirae*, which forms a large supramolecular protein complex (total molecular weight: ~700,000), physiologically transports Na^+ and Li^+ across a hydrophobic lipid bilayer. Stabilization of these cations in the binding site has been discussed based on the X-ray crystal structures of a membrane-embedded domain, named the K-ring (Na^+ and Li^+ bound forms). Here, we applied ATR-FTIR spectroscopy on this large protein complex for the first time, and we measured sodium or lithium ion binding-induced difference infrared spectra of the intact V-ATPase with sufficient amount of hydration at physiological temperature.[1] The results suggest that binding of sodium or lithium ion induces the deprotonation of Glu139, resulting in a hydrogen-bonding change around a tyrosine residue and a little conformational change in the K-ring. These structural changes, especially the deprotonation of Glu139, are considered to be important for reducing energetic barriers to the transport of the cations across membranes.

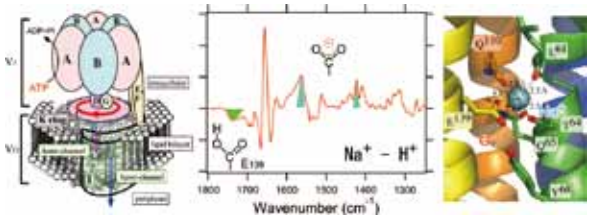

Figure 1. (left) Schematic structure of whole V-ATPase protein complex (center) Sodium ion binding induced difference infrared spectrum. The green and cyan colored bands are assigned to protonated and deprotonted carboxylic acid residues of Glu139 in K-ring, respectively. (right) Sodium ion binding site revealed by X-ray crystallography on K-ring.

2. Ion-Selective Mechanism of Mg^{2+} Transporter MgtE Studied by ATR FT-IR

Mg^{2+} is the most abundant divalent cation in living cells. MgtE is one of the Mg^{2+} transporters that function in the homeostasis of the intracellular Mg^{2+} concentration, in which the cytosolic domain acts as a 'Mg^{2+} sensor' and the transmembrane (TM) domain works as a 'pore.' Under high-intracellular Mg^{2+} conditions (> 10 mM), Mg^{2+} binding to the MgtE stabilizes a "closed" state and shuts down the Mg^{2+} transport from the extracellular part. In contrast, under low-intracellular Mg^{2+} conditions (< 1 mM), the MgtE is in equilibrium between the "closed" and "open" states, which can transport Mg^{2+}. In addition, MgtE is a highly Mg^{2+}-selective channel and unable to transport other divalent-cations including near-cognate Ca^{2+}. We performed the perfusion-induced difference ATR-FTIR measurements to understand the molecular mechanism underlying the Mg^{2+}-selectivity of MgtE.

The difference FTIR spectrum between the magnesium- and calcium-binding forms showed the bands between 1430 and 1380 cm^{-1} which are assigned to carboxylate groups. The COO^- bands in the presence of Mg^{2+} were observed in the higher wavenumber than in the presence of Ca^{2+}, indicating weaker interaction of the carboxylate groups with Mg^{2+} than with Ca^{2+}. This result contradicts the stronger electrostatic interaction of fully dehydrated Mg^{2+} with COO^- group compared with Ca^{2+}. On the other hand, the titration experiment for the closed states revealed that there are some binding sites to which Mg^{2+} binds ~40 times higher affinity than Ca^{2+} binds. The Mg^{2+}-binding site composed of the Asp432 in the TM domain (Mg1 site) is a plausible candidate based on our mutagenesis and protease protection experiments. These results suggest that the higher affinity for Mg^{2+} is due to the size of the cavity that can accommodate the hydrated Mg^{2+} rather than its charge intensity.

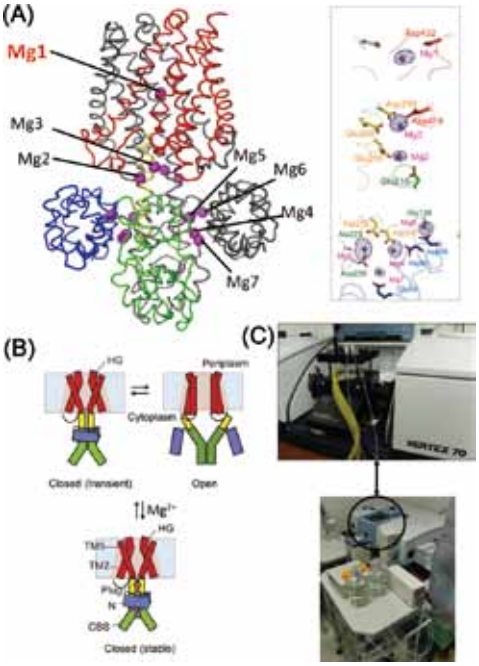

Figure 2. (A) X-ray crystal structure of MgtE in the closed state. *Inset*: Divalent cation binding sites. (B) Proposed gating mechanism of MgtE. (C) Pictures of the perfusion-induced difference ATR-FTIR measurements system.

3. Surface Enhanced Infrared Spectroscopy for Membrane Proteins in a Single Sheet of Lipid Bilayers

Monitoring structural changes of membrane proteins in a single sheet of lipid bilayer has been considered to be an ideal measurement method, while it is probably hard to detect tiny spectral change of the single-layered proteins. A graduate student, Mr. Hao Guo, has been struggling to establish the method. He got an award from SOKENDAI in advance of publication. In the near future, we will accomplish this challenging subject.

4. Development of a Microfluidic Device to Monitor Biological and Chemical Reactions

Real-time observation is one of the powerful techniques to understand the molecular mechanisms of the self-organization and molecular association. The solution mixing technique realizes many reaction fields for biological and chemical reactions by changing the buffer condition and can be combined with spectroscopic equipment easily. However, conventional mixing techniques limit their targets because of the large consumption of the sample. We are developing a novel microfluidic device and trying to reduce the sample consumption to ~1/1000. In addition, we aim to achieve the time-resolution of several microseconds by combining with microscopic techniques.

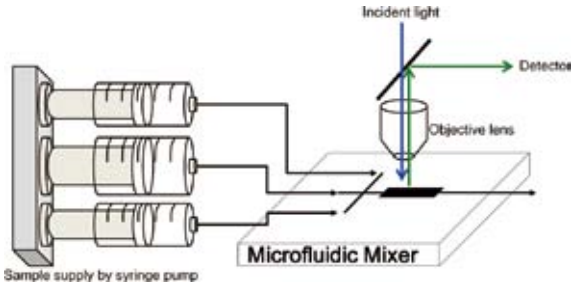

Figure 3. Schematic drawing of the microfluidic mixer.

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References

1) Y. Furutani, T. Murata and H. Kandori, *J. Am. Chem. Soc.* **133**, 2860–2863 (2011).
2) J. Sasaki, H. Takahashi, Y. Furutani, H. Kandori and J. L. Spudich, *Biophys. J.* **100**, 2178–2183 (2011).

Award

GUO, Hao; FY2011 (the 2nd) Sokendai President's Award.

* carrying out graduate research on Cooperative Education Program of IMS with Nagoya Institute of Technology