Investigation of Molecular Mechanisms of Transporters and Receptors in Membrane by Using Stimulus-Induced Difference FT-IR Spectroscopy

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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 one of 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 spectroscopy which is sensitive to the structural and environmental changes of organic and bio-molecules.

Our research group was launched in 2009 and has been setting up experimental equipments.

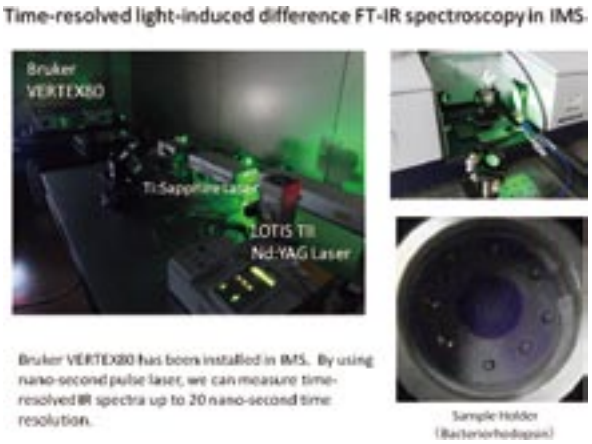

Figure 1. Light-induced difference FT-IR measurement system.

1. Time-Resolved FT-IR Spectroscopy Detecting O–H and O–D Stretching Vibrations of Light-Driven Proton and Chloride-Ion Pump Proteins

Bacteriorhodopsin (bR) is one of well known light-driven proton pump protein, which has an all-*trans* retinal as a chromophore. Upon the light absorption, photo- isomerization of the retinal occurs from the all-*trans* to 13-*cis* form in less than one picosecond, followed by a cyclic reaction that comprises a series of intermediates, called as the K, L, M, N, and O states, back to the bR ground state (BR).

Hydration level of bR film samples are optimized by dropping the deuterium glycerol/water drops (20%v/v) around the sample. This sample preparation allowed us to collect the accurate light-induced difference FT-IR spectra (3850 to 1000 cm^{-1}) including the X–H and X–D stretching regions, corresponding to the structural changes in the hydrophobic and hydrophilic parts, respectively. By analyzing these spectral changes, real-time hydrogen-bonding changes of internal water molecules and protein moiety of BR will be elucidated, leading to better understand of the light-driven proton pumping mechanism of BR.

Dr. Kimura joined our group in Dec. 2009 and started to measure time-resolved infrared spectra of a light-driven chloride-ion pump, *pharaonis* halorhodopsin (*p*HR). The previous low-temperature FT-IR measurement revealed that the specific interaction among a chloride ion, the protonated Schiff base and internal water molecules changes upon the formation of K, L_1 and L_2 intermediates [Shibata *et al.*, *Biochemistry* **44**, 12279 (2005).] However, the structures in the later intermediates, such as N, O, and *p*HR' are still unclear. In this study, we applied time-resolved FT-IR difference spectroscopy on *p*HR at 12.5 μs time resolution. As a result, we found that the H/D unexchangeable X–H groups showed two negative peaks at 3289 and 3315 cm^{-1} in amide A region, with different decay kinetics. The former band was decayed with the time

constant of 4.2 ms, but the latter decay was fitted with the two time constants of 700 μs and 6.2 ms. The faster and slower phases correspond to the conformational changes from L_2 to [N ↔ O] states accompanying chloride release and from [N ↔ O] to *p*HR' involving chloride uptake, respectively.

2. Perfusion-Induced Difference FT-IR Spectroscopy Investigating Ion Binding Sites of Membrane Proteins

Recently, it has been demonstrated that stimulus-induced attenuated total reflection (ATR) FT-IR spectroscopy is one of the promising techniques for the investigation of the molecular mechanism of membrane proteins.

ATR FT-IR spectroscopy is a useful technique to obtain infrared spectra of membrane proteins immersed in aqueous solution. By exchanging buffer with and without salts, the difference spectra between the two conditions provide the structural information relating to the interaction change between membrane proteins and ions, especially around the ion binding site. We first applied this technique to a component of a flagellar motor protein complex, PomA/PomB, which forms ion channels passing Na^+ ions to drive the rotation of flagella. We revealed that Asp24 is deprotonated upon binding of Na^+ ion to PomA/PomB and is involved in an Na^+ pathway, which probably reduces the energetic barrier formed in the inside of the hydrophobic channel.[1)]

Application of this ATR FT-IR to other membrane proteins, such as an ion channel (KcsA) and a transporter protein (V-ATPase), started before coming to IMS. It has been succeeded to measure the difference infrared spectra between the conditions of several types of ions and pH. Based on these spectra, the molecular mechanism of recognition and transportation of ions will be discussed in near future.

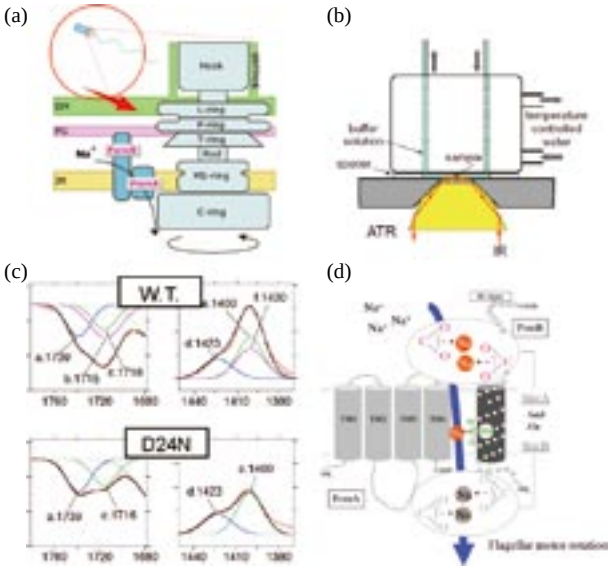

Figure 2. Sodium-ion binding induced difference FT-IR spectroscopy for a flagellar motor protein. (a) Schematic figure of supramolecular complex of flagellar motor. (b) A sample holder for perfusion-induced difference FT-IR spectroscopy. (c) COOH and COO^- vibrations observed upon dissociation and binding of sodium ions, respectively. One of them was assigned to Asp24. (d) The proposed mechanism for sodium-ion flux through the PomA/PomB complex.

In Apr. 2010, a graduate student, Mr. Guo, joined our group and started to construct an ATR FT-IR measurement system with surface-enhanced technique. He has succeeded to make thin gold films by vapor deposition method and observed the AFM images. The surface enhancement of raman scattering from pyridine solution was confirmed by raman microscopy. In future, we will make a thin gold film on an ATR crystal, attach membrane proteins on the surface and measure the light and perfusion-induced difference FT-IR spectra.

3. FT-IR Studies for Revealing Molecular Mechanisms of Visual Rhodopsins

In our vision, rhodopsin works in the dim light and cone pigments in the daylight. The former is specialized for scotopic vision and the latter is for discriminating color.

We found that Thr118-OH group of bovine rhodopsin is utilized for an internal molecular probe to investigate protein fluctuation.[2)] By monitoring a slow hydrogen/deuterium exchange (HDX) of the Thr118-OH group under native conditions and analyzing the data through the precise mathematical statistics, we proposed a quantitative two-step model in which the dark activation of rhodopsin is triggered by thermal isomerization of the retinal in a transiently opened conformation.

The red and green cone pigments from primate were heterologously expressed in HEK293 cells, purified by affinity chromatography, and then reconstituted in phosphatidylcholine (PC) liposomes. By using low-temperature FT-IR spectroscopy, the structural changes upon the retinal isomerization were analyzed and the molecular mechanism of red and green color discrimination was discussed.[3)]

Acknowledgements

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References

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