Investigation of Molecular Mechanisms of Channels, Transporters and Receptors in Cell Membrane

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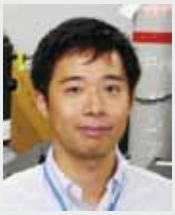

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Membrane proteins are important for homeostasis of living cells, which work as ion channels, transporters, various types of chemical and biophysical sensors, and so on. These proteins are considered as important targets for biophysical studies.

Our main goal is to clarify molecular mechanisms of channels, 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 bio-molecules.

1. O–H Stretching Vibrations of Dangling Bonds of Water Molecules in *pharaonis* Halorhodopsin Studied by Time-Resolved FTIR Spectroscopy

Ion transportation via the chloride ion pump protein *pharaonis* halorhodopsin (*p*HR) occurs through the sequential formation of several intermediates during the photocyclic reaction. Although the structural details of each intermediate state have been studied, the role of water molecules in the translocation of chloride ions inside the protein at physiological temperatures remains unclear. To analyze the structural dynamics of water inside the protein, we performed time-resolved Fourier transform infrared (FTIR) spectroscopy under H_2O or $H_2{}^{18}O$ hydration and successfully assigned water O–H stretching bands.[1)] We found that a dangling water band at 3626 cm^{-1} in *p*HR disappears in the L_1 and L_2 states. On the other hand, relatively intense positive bands at 3605 and 3608 cm^{-1} emerged upon the formation of the N and O states, respectively, suggesting that the chloride transportation is accompanied by dynamic rearrangement of the hydrogen-bonding network of the internal water molecules in *p*HR (Figure 1) .

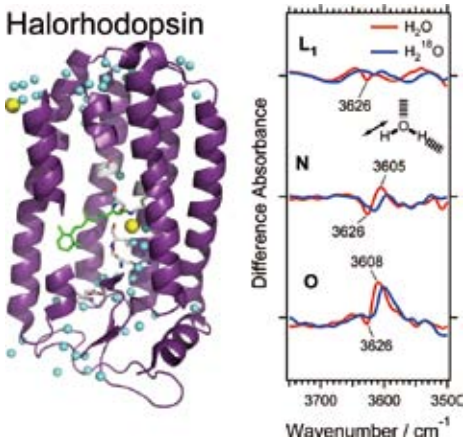

Figure 1. X-ray crystal structure of halorhodopsin (left). The O–H stretching bands of dangling bonds of water molecules inside the protein(right). The figure is adapted with permission from ref. 1. Copyright 2012 American Chemical Society.

2. Perfusion-Induced ATR-FTIR Spectroscopy for Studying Ion-Protein Interactions of a Potassium Ion Channel, KcsA

The potassium channel is highly selective for K^+ over Na^+,

and the selectivity filter binds multiple dehydrated K^+ ions upon permeation. Here, we applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to extract ion-binding-induced signals of the KcsA potassium channel at neutral pH.[2] Shifts in the peak of the amide-I signal towards lower vibrational frequencies were observed as K^+ was replaced with Na^+ (Figure 2). These ion species-specific shifts deduced the selectivity filter as the source of the signal, which was supported by the spectra of a mutant for the selectivity filter (Y78F). The difference FTIR spectra between the solution containing various concentrations of K^+ and that containing pure Na^+ demonstrated two types of peak shifts of the amide-I vibration in response to the K^+ concentration. These signals represent the binding of K^+ ions to the different sites in the selectivity filter with different dissociation constants (K_D = 9 or 18 mM).

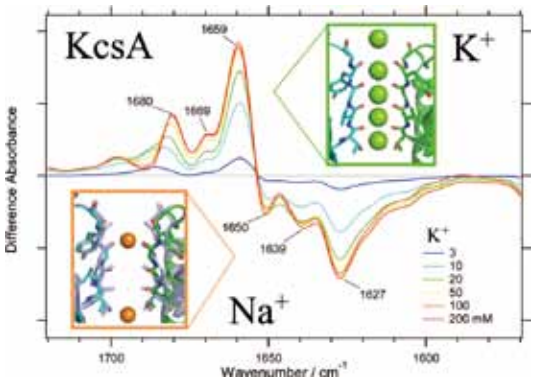

Figure 2. The K^+-minus-Na^+ difference spectra measured with different K^+ concentration. The insets show the X-ray crystal structures of KcsA. The figure is reprinted with permission from ref. 2. Copyright 2012 American Chemical Society.

3. Development of the Measurement Condition of Surface-Enhanced Infrared Absorption Spectroscopy on Membrane Proteins

Surface-enhanced infrared absorption with attenuated total reflection (ATR-SEIRA) is a powerful tool for exploring molecular mechanisms of membrane proteins at the monolayer level. However, the band intensity, position, and direction can be largely influenced by the presence of a thin gold film, as observed for small molecules existing in close proximity to the surface. Here we investigated influence on the band shapes of an α-helical membrane protein, *p*HR, attached on the gold surface through a complex formation between a six-histidines tag and a Ni-nitrilotriacetic acid (Ni-NTA) linker.[3] Normal, bipolar, and inverted shapes of amide-I and -II bands were observed with an increase in film thickness, although *p*HR molecules would locate relatively far from the surface. The physical origin of this interesting phenomenon has been identified by changing incident angle, polarization, and film deposition rate. We find the observed absorption anomalies are due to the influence of perpendicularly polarized light. Furthermore, it is shown that the band shapes are normal below the percolation threshold, and bipolar ones occur when an anomalous absorption by the films is strong, while the inverted ones develop with films in which surface scattering is predominant.

4. Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode

Attenuated total reflectance (ATR)-FTIR spectroscopy has been widely used to probe protein structural changes under various stimuli, such as light absorption, voltage change, and ligand binding, in aqueous conditions. Time-resolved measurements require a trigger, which can be controlled electronically; therefore, light and voltage changes are suitable. Here we developed a novel, rapid buffer-exchange system for time-resolved ATR-FTIR spectroscopy to monitor the ligand- or ion-binding reaction of a protein.[4] By using the step-scan mode (time resolution; 2.5 ms), we confirmed the completion of the buffer-exchange reaction within ~25 ms; the process was monitored by the infrared absorption change of a nitrate band at 1350 cm^{-1} (Figure 3). We also demonstrated the anion-binding reaction of a membrane protein, *p*HR, which binds a chloride ion in the initial anion-binding site near the retinal chromophore. The formation of chloride- or nitrate-bound *p*HR was confirmed by an increase of the retinal absorption band at 1528 cm^{-1}. It also should be noted that low sample consumption (~1 μg of protein) makes this new method a powerful technique to understand ligand–protein and ion–protein interactions, particularly for membrane proteins.

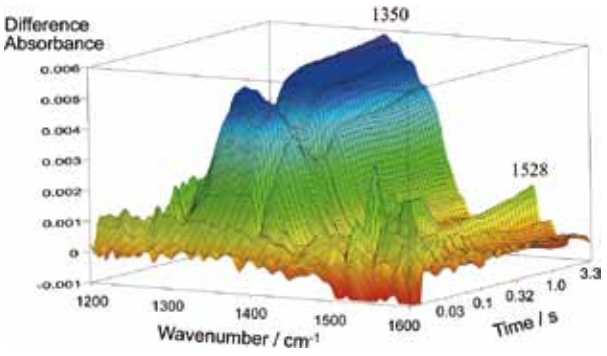

Figure 3. The time-resolved FTIR spectra of the rapid-buffer exchange reaction of pHR. The figure is reprinted with permission from ref 4. Copyright 2013 The Biophysical Society of Japan.

References

1) Y. Furutani *et al.*, *J.Phys. Chem. Lett.* **3**, 2964–2969 (2012).
2) Y. Furutani *et al.*, *J. Phys. Chem. Lett.* **3**, 3806–3810 (2012).
3) H. Guo *et al.*, *Chem. Phys.* **419**, 8–16 (2013).
4) Y. Furutani *et al.*, *Biophysics* **9**, 123–129 (2013).