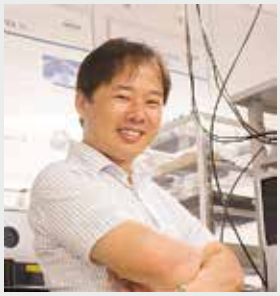
Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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



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Education
1999 B.S. Kyoto University
2004 Ph.D. Kyoto University

Professional Employment
2003 JSPS Research Fellow
2004 JSPS Postdoctoral Fellow
2006 Assistant Professor, Nagoya Institute of Technology
2009 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies
2011 JST-PRESTO Researcher (concurrent post) (–2015)
2018 Associate Professor, Nagoya Institute of Technology

Awards
2012 Morino Foundation for Molecular Science
2013 The 2013 Young Scientist Awards of the Japan Society for Molecular Science

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Membrane proteins are important for homeostasis and signaling 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. Our main goal is to clarify molecular mechanisms underlying functions of the channels, transporters and receptors mainly by using stimulus-induced difference infrared spectroscopy, which is sensitive to the structural and environmental changes of bio-molecules.

We applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to extract ion-binding-induced signals of various kinds of membrane proteins. For example, KcsA is a potassium channel, which is highly selective for K^+ over Na^+, and the selectivity filter binds multiple dehydrated K^+ ions upon permeation. Shifts in the peak of the amide-I signals towards lower vibrational frequencies were observed as K^+ was replaced with Na^+ (Figure 1). These vibrational modes give us precise structural information of the selectivity filter. Moreover, by changing concentrations of K^+ in buffer solutions, we can estimate affinity of the selectivity filter for K^+ ions.

Recently, we have developed a rapid-buffer exchange apparatus for time-resolved ATR-FTIR spectroscopy, which can be utilized for studying dynamics of structural transition in membrane proteins.

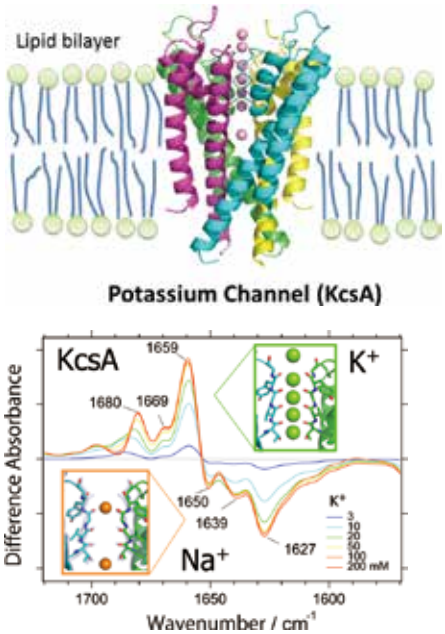

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

Selected Publications

- Y. Furutani *et al.*, "ATR-FTIR Spectroscopy Revealed the Different Vibrational Modes of the Selectivity Filter Interacting with K^+ and Na^+ in the Open and Collapsed Conformations of the KcsA Potassium Channel," *J. Phys. Chem. Lett.* **3**, 3806–3810 (2012).
- Y. Furutani *et al.*, "Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode," *Biophysics* **9**, 123–129 (2013).

1. Ion–Protein Interactions of MgtE Magnesium Channel with Magnesium or Calcium Ions and Its Implication for the Ion Selectivity[1)]

Magnesium ion (Mg^{2+}) is vital for living systems and utilized for various biological processes. Calcium ion (Ca^{2+}) is also important as a second messenger inside the cell. Thus, the selective permeation of Mg^{2+} is not only prerequisite for homeostasis of internal Mg^{2+} concentration and also for avoiding unintended induction of calcium signaling.

MgtE is an ion channel highly selective to Mg^{2+}. The crystal structure of MgtE showed a dimeric structure with transmembrane domain and two cytosolic domains [N- and cystathionine-β-synthase (CBS) domains]. The transmembrane domains constitute a pore with a central cavity which is important for the ion selectivity to Mg^{2+}. In addition, the crystal structure of the transmembrane region with higher resolution revealed that a Mg^{2+} ion exists with hydrated water molecules in the cavity.

To elucidate the molecular mechanisms of the ion selectivity for Mg^{2+} of MgtE in more detail, we applied ion-exchange induced difference FTIR spectroscopy with an aid of computational methods. By changing electrolyte solution containing Mg^{2+} with that of Ca^{2+}, we obtained an infrared difference spectrum of MgtE which contains molecular information of the ion–protein interactions with Mg^{2+} or Ca^{2+}. Comparing the difference spectra of several site-directed mutant proteins of MgtE, we assigned antisymmetric and symmetric COO^- vibrations of Asp432 which was found to be crucial for the ion selectivity of MgtE.

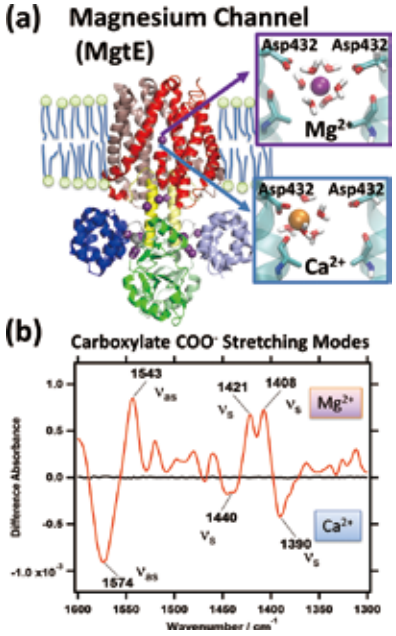

Figure 2. (a) The X-ray crystal structure of MgtE and snap shots of molecular dynamics simulation with Mg^{2+} or Ca^{2+} ion. (b) The ion-exchange induced difference infrared spectrum of MgtE in the carboxylate COO^- stretching region.

Moreover, from systematic measurements with the different ion concentrations, we estimated the dissociation constant relating to the central cavity and found that the value is much lower for Mg^{2+} (~0.3 mM) compared to that for Ca^{2+} (~80 mM). The difference of affinity is well consistent with the high selectivity for Mg^{2+} of MgtE elucidated by electrophysiological and biological methods. Difference in frequency of COO^- stretching vibrations of Asp432 in the central cavity suggests that ion–protein interactions with Mg^{2+} and Ca^{2+} are different from each other. To get more information about energetics of the ion–protein interactions and dynamics, we applied molecular dynamics simulation and normal mode analysis with quantum chemical calculation on MgtE with hydrated Mg^{2+} or Ca^{2+} in the cavity. We found that Mg^{2+} is more stable with hydrated configuration at the center between two Asp432 residues, but Ca^{2+} is easily captured by either of the residues and forms the direct interaction. In this way, our experimental and computational approach provided new insights for the ion selectivity of MgtE.

2. Detection of Ligand- or Light- Induced Structural Changes in G Protein-Coupled Receptors Using ATR-FTIR Spectroscopy

My group has developed attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to trace and reveal structural features of membrane proteins involved in various important biological functions. Based on IR spectral changes, we have reported how functional properties of ion channels (an ATP-sensitive P2X receptor and a potassium channel TWIK1) are regulated. In order to extend the applicability of ATR-FTIR spectroscopy to other important membrane proteins, we tried to measure IR spectral changes of G protein-coupled receptors (GPCRs) upon agonist-binding or light-absorption since GPCRs are involved in many important cellular processes such as neuro- transmission, hormone perception, vision and olfaction.

Besides ligand-binding GPCR, we also tried to measure IR spectral changes of light-sensitive GPCR opsin. We previously reported that an opsin in the brain of a zooplankton is activated by UV light and inactivated by visible light. Through the ATR-FTIR techniques, IR spectral changes of the opsin upon activation and inactivation were successfully detected. Time-resolved IR measurements of the opsin as well as ligand-binding GPCR would reveal how signal reception (UV light absorption for the opsin and ligand-binding for GPCRs) evokes conformational changes in the GPCRs toward activated states.

Reference

1) T. Kimura, V. A. Lorenz-Fonfria, S. Douki, H. Motoki, R. Ishitani, O. Nureki, M. Higashi and Y. Furutani, *J. Phys. Chem. B* **122**, 9681–9696 (2018).

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