Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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1999 B.S. Kyoto University
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Professional Employment
2003 JSPS Research Fellow
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Awards
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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.

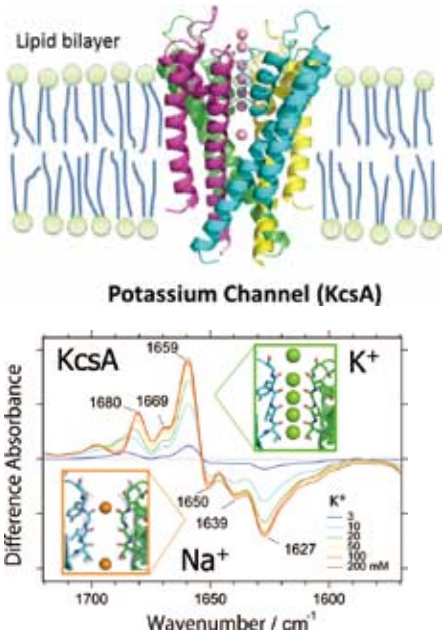

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

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. Formation of Host–Guest Complexes on Gold Surface Investigated by Surface-Enhanced IR Absorption Spectroscopy[1)]

Surface-enhanced infrared absorption with attenuated total reflection (ATR-SEIRA) is a powerful tool for studying molecular systems at the monolayer level.

Ionophores capture guest ions selectively and carry them across interfaces efficiently. One of crown ethers, 18-crwon-6 (18C6) is one of well known ionophores for a potassium ion. Molecular mechanisms of the ion selectivity of 18C6 have been investigated by SEIRA spectroscopy as a cooperative research with Assoc. Prof. Inokuchi in Hiroshima University.

Thiol derivatives of 18C6 [2-(6-mercaptohexyloxy)methyl-18-crown-6 (18C6-C_1OC_6-SH) and 2-(mercaptomethyl)-18-crown-6 (18C6-C_1-SH)] were synthesized and chemisorbed on a gold surface (Figure 2). Aqueous solutions of MCl salts (M = alkali metals) were put on it to form M^+•18C6-C_1OC_6 and M^+•18C6-C_1 complexes. Infrared spectra of these complexes in the 2000–900 cm^{-1} region were obtained by SEIRA spectroscopy.

As a result, the SEIRA spectra of 18C6 with K^+ are very similar to those with Rb^+ and Cs^+, but largely different from those with Li^+ and Na^+. Moreover, it was demonstrated that the affinity for K^+ is higher than those for other alkali cations. Obtained results proved that SEIRA spectroscopy is a powerful method to examine the structure of host-guest complexes and the solvent effect on them in solutions.

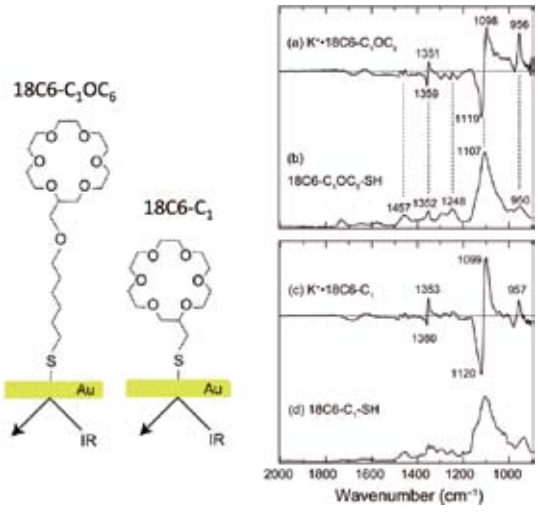

Figure 2. (left) Schematic figures of crown ethers immobilized on the gold surface through a S–Au bond. (right) SEIRA spectra of crown ethers 18C6-C1OC6 (a) and 18C6-C1 (c) recorded after addition of 0.1 M KCl solution. The absorption spectra of the crown ethers (b) and (d) were recorded by a conventional ATR-FTIR method. This figure is reproduced from ref. 1.

2. Deformation of β-Sheet Structures of the GroEL Apical Domain Induced at Sub-Micellar Detergent Condition[2)]

SEIRA spectroscopy is a useful tool to analyze protein structure as well. GroEL is a chaperonin which refolds denatured proteins with a cofactor GroES by utilizing hydrolysis energy of ATP. Dr. Jin Chen, who was an IMS research assistant professor in Prof. Kuwajima's Group in Okazaki Institute for Integrative Bioscience, studied the property of GroEL for formation of protein nanofibers at sub-micellar detergent condition. To understand the molecular mechanism of the fiber formation, SEIRA analysis on the GroEL apical domain was performed (Figure 3).

The data clearly showed SDS-dependent deformation of β-sheet structures in the GroEL apical domain, which would promote the formation of the nanofiber in the later stage.

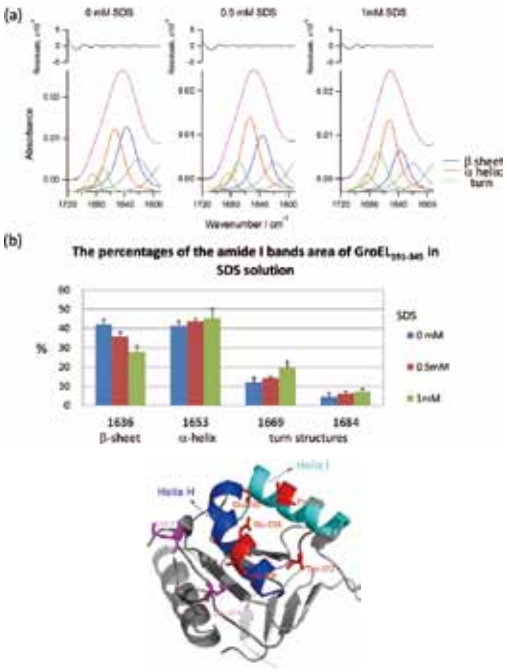

Figure 3. (a) The SEIRA spectra of GroEL apical domain recorded in SDS solution (0, 0.5, and 1 mM). The amide I bands are analyzed by band fitting method. (b) The effect of SDS on the secondary structures of GroEL apical domain. (c) The X-ray crystal structure of GroEL apical domain. This figure is adapted from ref. 2.

References

1) Y. Inokuchi*, T. Mizuuchi, T. Ebata, T. Ikeda, T. Haino, T. Kimura, H. Guo and Y. Furutani, *Chem. Phys. Lett.* **592**, 90–95 (2014).
2) J. Chen*, H. Yagi, Y. Furutani, T. Nakamura, A. Inaguma, H. Guo, Y. Kong and Y. Goto, *Sci. Rep.* **4**:5614 (2014).
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Awards

FURUTANI, Yuji; The 2013 Young Scientist Awards of the Japan Society for Molecular Science.
FURUTANI, Yuji; The 1st BIOPHYSICS Editors' Choice Award (2014).

* IMS International Internship Program