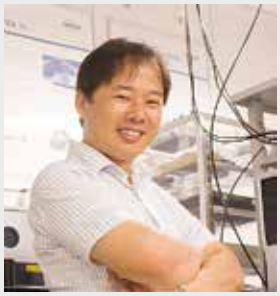
Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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



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1999 B.S. Kyoto University
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Professional Employment
2003 JSPS Research Fellow
2004 JSPS Postdoctoral Fellow
2006 Assistant Professor, Nagoya Institute of Technology
2009 Associate Professor, Institute for Molecular Science
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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.

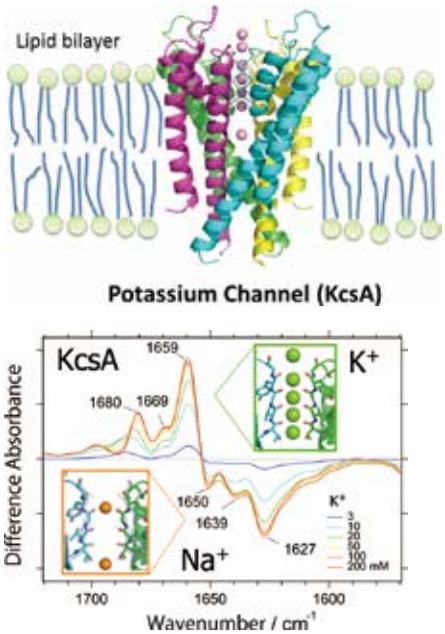

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

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. Molecular Characteristics of a Mammalian Photoreceptive Protein, Melanopsin for Non-Visual Function[1)]

Animals use external light signals not only for vision but also for "non-visual" functions such as regulation of biological clock. In particular, mammals including human receive ambient light through retina, leading to photoentrainment of the circadian clock and pupil responses. It had been thought that visual cells (rods and cones) are only photoreceptor cells in mammalian retina, but recent studies have shown that small population of retinal ganglion cells are also photoreceptive and play important roles in the non-visual photoreception. Since the intrinsically photoreceptive retinal ganglion cells (ipRGCs) show extremely low photosensitivity (less than 1/10,000-fold sensitivity of visual cells), mammals can detect condition of ambient light in a wide dynamic range by using ipRGCs as well as visual cells. Thus, lowering the photosensitivity of ipRGCs is important for non-visual photoreception in mammals.

ipRGCs express a photoreceptive protein melanopsin. Like visual pigments in rods and cones, melanopsin is a member of the opsin family, and it consists of a protein moiety with seven transmembrane α-helices and the chromophore retinal. Interestingly, the amino acid sequence of melanopsin is more similar to that of invertebrate visual pigment rather than to that of vertebrate visual pigment (Figure 2a). In this context, we speculated that mammalian melanopsins possess some molecular characteristics contributing to the low photosensitivity of ipRGCs. We thus compared biochemical, spectroscopic and electrophysiological properties of mouse and human melanopsins with those of closely related invertebrate melanopsin and visual pigment.

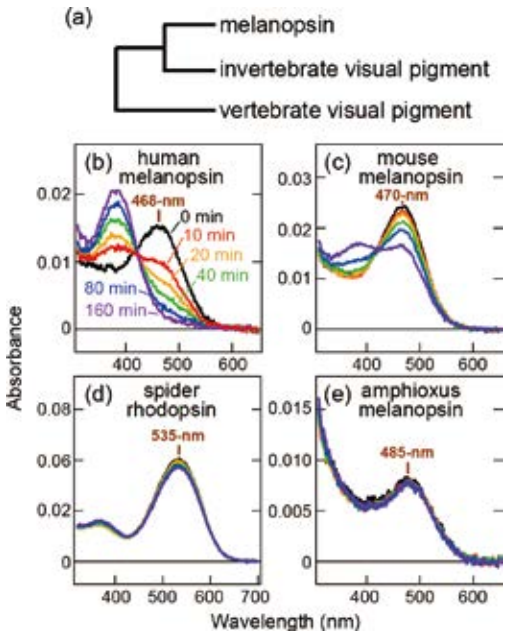

Figure 2. (a) Schematic representation of phylogenetic relationship of melanopsin, invertebrate visual pigment and vertebrate visual pigment. (b)–(e) Absorption spectra showing time-dependent loss of absorbance in the visible region for human melanopsin (b), mouse melanopsin (c), jumping spider rhodopsin, an invertebrate visual pigment (d), and an invertebrate amphioxus melanopsin (e). Panels (b)–(e) are adopted from ref. 1.

We expressed mouse and human melanopsin in mammalian cultured cells, and purified them through immuno-affinity chromatography. The purified proteins were kept at 37 °C in the dark, and subsequent spectral changes were recorded. During the incubation, the mammalian melanopsins showed time-dependent loss of absorbance at ~470-nm, indicating that they spontaneously hydrolyze the Schiff base linkage with the retinal (Figure 2, b and c). In contrast, such a hydrolysis was not observed in invertebrate melanopsin and visual pigment, both of which are closely related to mammalian melanopsins (Figure 2, d and e). Interestingly, human melanopsin showed the hydrolysis much faster (~10-fold) than mouse one whereas their sequences are very similar (~80% identity). Electrophysiological analyses using *Xenopus* oocytes expressing human or mouse melanopsin confirmed that the faster retinal release in human melanopsin occurs in cells, too.

These results indicate that mammalian melanopsins can loss their photoreceptive ability by spontaneous hydrolysis and release of the retinal chromophore. This characteristic would have an effect to lower photosensitivity of melanopsin-expressing cells (Figure 3). Our findings suggest that molecular characteristics of mammalian melanopsin are tuned for their non-visual photoreception. In addition, the further destabilized retinal attachment in human melanopsin would result in further lowered photosensitivity of human ipRGCs. This "enhanced" characteristic of human melanopsin might reflect adaptation to bright environment humans live.

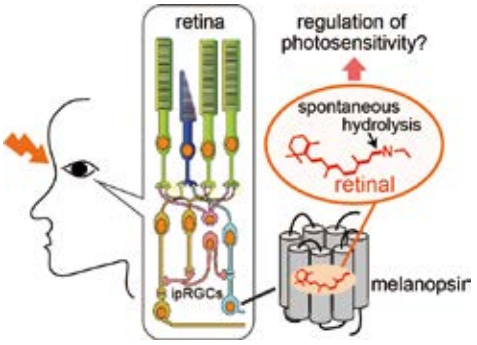

Figure 3. Our model illustrating how molecular characteristics of melanopsin contributes to non-visual photoreception in mammals. The spontaneous hydrolysis would play an important role in lowering photosensitivity of the cells.

Reference

1) H. Tsukamoto*, Y. Kubo, D. L. Farrens, M. Koyanagi, A. Terakita and Y. Furutani, *J. Biol. Chem.* **290**, 27176–27187 (2015).

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* IMS International Internship Program