【Grant-in-Aid for Specially Promoted Research】

Science and Engineering

Title of Project： Elucidation of the mechanism of rhodopsin functions for optogenetics

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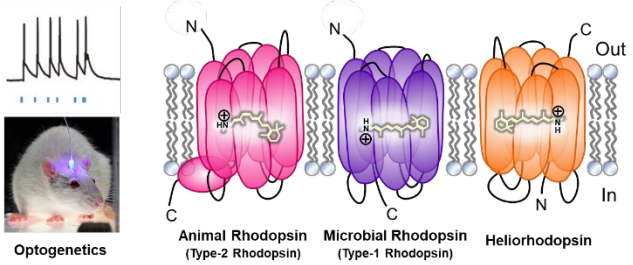
【Purpose and Background of the Research】

Rhodopsins convert light into signal and chemical energy by use of the chromophore molecule, retinal. Animal and microbial rhodopsins possess 11-*cis* and all-*trans* retinal, respectively, to capture light in seven transmembrane α-helices, and photoisomerizations into all-*trans* and 13-*cis* forms, respectively, initiate each function. While animal rhodopsins are G-protein coupled receptors (GPCRs) that activate a G-protein transducin by light, function of microbial rhodopsins is highly diverse. Many microbial rhodopsins are able to transport ions in a passive or an active manner, while light sensor and light-activated enzyme functions have been also known. In addition, we recently discovered heliorhodopsins (HeRs) that have little sequential homology with the known microbial rhodopsins.



In this project, we will tackle three challenging questions on animal and microbial rhodopsins.

First, we try to determine the structures of color visual pigments. We discriminate various colors using three pigments, red, green and blue, whose structural study has been solely performed by our IR spectroscopy. The structure of color visual pigments, together with structural dynamics by IR study, will reveal the mechanism of our color vision.

Second, we will reveal molecular mechanisms of new type-1 microbial rhodopsins such as light-driven sodium pump, inward proton pump, channelrhodopsin with high photosensitivity, and enzyme rhodopsins. By spectroscopic and electrophysiological methods, we will elucidate how multiple functions emerge from common structure. In addition, we will search new functions.

Third, heliorhodopsin (HeR) is categorized into microbial rhodopsin, whose function is unknown. Comprehensive spectroscopic, electrophysiological, biochemical and physiological analysis will be applied to HeRs from eubacteria, archaea, eukaryotes and viruses, eventually leading to elucidation of the function of HeRs.

【Research Methods】

Highly accurate difference FTIR spectroscopy has been a key method in our rhodopsin research. In this project, various rhodopsins will be prepared using heterologous expression systems such as E. coli, Pichia pastoris, insect cell, and mammalian cells. Purified proteins will be studied spectroscopically, biochemically and electrophysiologically. Specific methods for three goals are described below.

[1] Animal rhodopsins: [Challenge: Structural determination of color visual pigments] Thermally stabilized primate color visual pigments will be crystallized, to which X-ray crystallography is applied.

[2] Novel type-1 microbial rhodopsins: [Challenge: Understanding the origin of diversity] Various biophysical methods will be applied to these proteins.

[3] Heliorhodopsins: [Challenge: Identification of the function of heliorhodopsin] Comprehensive analysis will be applied to determine the function of heliorhodopsins.

【Expected Research Achievements and Scientific Significance】

Membrane proteins play important roles in our health, as shown for GPCR. Therefore, understanding of membrane proteins structure/function is highly demanded. In addition, rhodopsins are used as the tools for optogenetics, where understanding of molecular mechanism of rhodopsins is needed for the development of optogenetic tools. In view of application, light-driven cesium pump would be used to collect radioactive cesium from environment, and channelrhodopsin with high photosensitivity is possibly used for the gene therapy for visual reproduction.

【Publications Relevant to the Project】

- K. Katayama, Y. Furutani, H. Imai, H. Kandori: "An FTIR study of monkey green- and red-sensitive visual pigments" Angew. Chem. Int. Ed. 49, 891-894 (2010).
- A. Pushkarev, K. Inoue, S. Larom, J. Flores-Uribe, M. Singh, M. Konno, S. Tomida, S. Ito, R. Nakamura, S. P. Tsunoda, A. Philosof, I. Sharon, N. Yutin, E. V. Koonin, H. Kandori, O. Béjà: “A distinct abundant group of microbial rhodopsins discovered using functional metagenomics” Nature 558, 595-599 (2018).
- H. Kandori: “Structure/function study of photoreceptive proteins by FTIR spectroscopy” Bull. Chem. Soc. Jpn 93, 904-926 (2020).

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