【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Biology)

Title of Project : Structural and functional study of membrane proteins based on electron crystallography

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Research Area : Comprehensive fields

Keyword : Molecular and cellular neuroscience

【Purpose and Background of the Research】

Our long-term goal is to understand molecular mechanisms forming human ability and personality. Membrane proteins, such as water channels, ion channels, connexin, acetylcholine receptor and ATPases, are key components to almost all fundamental processes in the nervous system from the propagation of nerve impulses to the integration and storage of information. Together with various research techniques, structural analyses of membrane proteins by electron microscopy are thus important for understanding of brain function.

【Research Methods】

Electron microscopy provides biological information over a wide range of resolutions. At the higher resolution, the more insight can be obtained on the structure-function relationship of the biomolecules. To minimize radiation damage restricting resolution of biological structure analysis, we developed a low-dose imaging system as well as an original cryo-electron microscope (EM) equipped with a very stable, helium-cooled specimen stage. By electron crystallography and the cryo-EM, we are going to analyze structures of membrane proteins of water channels, ion channels, connexin and H^+,K^+-ATPase. We are particularly interested in AQP4, the predominant water channel in the brain. It has been implicated in serious human diseases, such as bipolar disorder and mesial temporal lobe epilepsy, constituting AQP4 as a membrane protein of high biomedical importance. Using electron crystallography, we determined the structure of AQP4 and found that it has an adhesive function. This finding led us to propose the concept of "adhennels", membrane channels with adhesive properties. We are currently working on the structure of several other adhennels, including gap junction channels (Cx26). We analyzed the structure of Cx26M34A mutant at 10Å resolution, which allowed us to propose the plug gating mechanism. We also solved the atomic structure of Cx26 by X-ray crystallography and proposed a model for a gating mechanism of transjunctional voltage. We are analyzing structure of Cx26M34A mutant at higher resolution for understanding complex gating mechanisms of gap junction channels. To solve channel structures, we are also studying bacterial voltage-sensitive Na^+-channels. After many trials, we established a cysteine double mutant and are growing two-dimensional crystal, while the crystal quality does not yet suffice for high-resolution structure analysis.

【Expected Research Achievements and Scientific Significance】

In this project, we will advance research in "structural physiology" by studying structure and function of AQP4, AQP6, Na^+-channels, gap junction channels and H^+,K^+-ATPase.

【Publications Relevant to the Project】

S. Maeda, S. Nakagawa, M. Suga, E. Yamashita, A. Oshima, Y. Fujiyoshi and T. Tsukihara; "Structure of the connexin-26 gap junction channel at 3.5 Å resolution." ***Nature***, **458,** 597-602 (2009).

K. Tani, T. Mitsuma, Y. Hiroaki, A. Kamegawa, K. Nishikawa, Y. Tanimura and Y. Fujiyoshi; "Mechanism of Aquaporin-4's Fast and Highly Selective Water Conduction and Proton Exclusion." ***J. Mol. Biol.***, **389,** 694-706 (2009).

【Term of Project】 FY2010-2014

【Budget Allocation】 167,100 Thousand Yen

【 Homepage Address and Other Contact Information】

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