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

Biological Sciences (Biology)

Title of Project： Studies in Structural Physiology of Channels

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Research Area：　Biology
Keyword：　Structural Biology

【Purpose and Background of the Research】

Recently, Cheng's group analyzed structure of TRP channels by single particle analysis (Liao et al., **Nature, 504**, 107-112, 2013) and we are developing a method named IBSA by which we can analyze structures of membrane proteins in lipid bilayers without crystallization utilizing a method similar to that used in single particle analysis. By improving electron crystallography and IBSA, we will challenge structural and functional studies of 6 types of membrane proteins; Gap junction channels, Acetylcholine receptor, water channels, Na^+ channels, H^+, K^+-ATPase and Tight junctions. Through structural and functional studies of these membrane proteins, we will advance the new research field of "Structural Physiology".

Based on crystallography, we published following papers; LHC (**Nature, 367**, 614-21, 1994), bR (**Nature, 389**, 206-11, 1997), AQPs (**Nature, 387**, 624-7, 1997, **Nature, 407**, 599-605, 2000, **Nature, 438**, 633-8, 205, **JMB, 355**, 628-39, 2006 etc), AChR (**Nature, 423**, 949-55, 2003, **JMB, 422**, 617-34, 2012 etc), Cx26 (**PNAS, 104**, 10034-39, 2007, **Nature, 458**, 597-602, 2009 etc), H^+,K^+-ATPase (**EMBOJ, 28**, 1637-43, 2009, **Nature C**, **2**, 155pp1-7, 2011, **PNAS, 109**, 18401-6, 2012 etc), Na^+ channels (**Nature, 409**, 1047-51, 2001, **JMB, 425**, 4074-88, 2013 etc), Claudin (**Science, 344**, 304-7, 2014, **Science, 347**, 775-8, 2015). Using crystallography as well as IBSA, we will analyze functional dynamic structures of 6 proteins.

【Research Methods】

The lipid environment is critically important for analyzing membrane protein structure, as exemplified by the fact that although the densities of water molecules in the channel were clearly discriminated by electron crystallography, even at a lower resolution of 2.8Å, the densities were blurred at a higher resolution of 1.8 Å by X-ray analysis (Fig. 1). The difference could be due to the different strengths of the helical dipole of two short helices of the water channel AQP4. The helical dipoles of these short helices observed in many channels are enhanced by the dielectric constant of the lipid bilayer. Therefore, structure analyses by electron crystallography and IBSA must evolve to advance the structural and functional studies of membrane proteins.

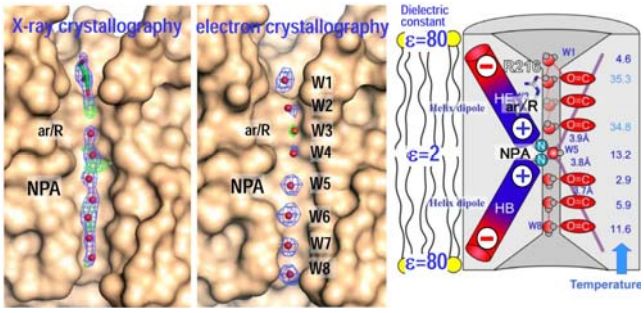

Fig. 1 Comparison between structure analyses by X-ray and electron crystallography. Only electron crystallography discriminated water densities in the channel. The electrostatic field formed by the helical dipole is important.

【Expected Research Achievements and Scientific Significance】

Advancing crystallography and IBSA, we will elucidate how channels work and regulate functions of the human body. We are intensively studying and advancing the interesting research field of Structural Physiology.

【Publications Relevant to the Project】

・H. Suzuki, T. Nishizawa, K. Tani, ・・・S. Tsukita, O. Nureki and Y. Fujiyoshi
Crystal structure of a Claudin provides insight into the architecture of tight junctions.
Science, 344, 304-307 (2014).

・Y. Saitoh, H. Suzuki, K. Tani, ・・・A. Tamura, S. Tsukita and Y. Fujiyoshi
Structural insight into tight junction disassembly by Clostridium perfringens enterotoxin.
Science, 347, 775-778 (2015).

【Term of Project】 FY2015-2019

【Budget Allocation】 138,500 Thousand Yen

【Homepage Address and Other Contact Information】

http:// www.cespi.nagoya-u.ac.jp/