

Crystal structure of channelrhodopsin light-gated cation channel

Channelrhodopsin (ChR) is a light-gated cation channel that conducts cations in a light-dependent manner. Because the inward flow of cations triggers neuron firing, neurons expressing ChRs can be optically controlled, even within freely moving mammals. Although ChR has now been broadly applied to neuroscience research, little is known about its molecular mechanisms. We determined the crystal structure of chimeric ChR at 2.3 Å resolution and revealed its molecular architecture, especially the cation-conducting pathway. The integration of structural and electrophysiological analyses provided insight into the molecular basis of the extraordinary function of ChR, and paved the way for the principled design of ChR variants with novel properties.

Organisms from bacteria to humans perceive light and use the information for visual and non-visual functions, including ATP synthesis and circadian rhythm. In most cases, light perception is mediated by proteins from the rhodopsin family, which consist of seven-transmembrane (7-TM) domains and covalently linked retinal chromophores. The first rhodopsin family protein was discovered in 1876 in frog retina by Franz Boll. Since that discovery, more and more rhodopsin family proteins have been isolated not only from animal eyes, but also from marine bacteria and even archaea living under extreme environmental conditions. So far, on the basis of their functions, the large number of rhodopsin family proteins can be divided into four distinct classes: photoisomerases, signal transducers, ion pumps, and the most recently discovered class, ion channels.

Channelrhodopsin (ChR) was originally isolated from tiny green algae, *Chlamydomonas Reinhardtii*, and identified as a light-gated cation channel in 2002 [1]. Beginning in 2005, it was found that ChRs could be expressed in mammalian neurons to mediate precise control of action potential firing in response to light pulses. ChRs have now been used to control neuronal activity in a wide range of animals, but little is known about the molecular mechanism of ChR. Although a rough helical arrangement was visible in the recently published electron microscopic (EM) structure of ChR at 6 Å resolution [2], amino acid positioning and insights into channel function remained completely lacking. High-resolution structure would be of enormous value, not only to enhance the understanding of the mechanism of this new class of rhodopsin family proteins, but also to guide the design of ChR variants with novel functions related to their spectrum, selectivity, and kinetics.

To solve the crystal structure of ChR, we expressed a chimeric ChR between ChR1 and ChR2 in Sf9 insect cells. The crystals were obtained in the lipidic mesophase, and the data set was collected at **BL32XU** beamline. A complete data set could be collected from a single tiny crystal (~50 μm) owing to the use of a 1-μm-wide, 15-μm-high microbeam and the helical data collection strategy [3]. The phase was determined by the multiple anomalous dispersion (MAD) method using mercury-derivatized crystals. As far as we know, this is the first example of the phase determination by MAD for a crystal obtained in the lipidic mesophase. We finally determined the crystal structure of ChR in the closed state at 2.3 Å resolution.

ChR is composed of an N-terminal extracellular domain, 7-TM domains connected by three cytoplasmic and extracellular loops, and a C-terminal intracellular domain (Fig. 1). Of particular note is that, as previously predicted from EM [2], ChR is tightly associated into a dimer via interfacial interactions between the N-domain, ECL1, TM3, and TM4 of each molecule. This result is somewhat of a surprise because all other known microbial rhodopsins form trimers or tetramers. This is the first example of microbial rhodopsin that forms a dimer conformation.

To further understand the ChR structure, we compared our ChR with the most well-studied microbial rhodopsin, bacteriorhodopsin (BR). Although

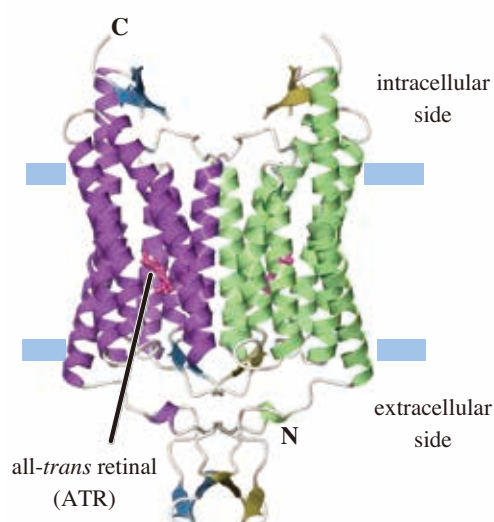


Fig. 1. Overall structure of chimeric ChR. Each protomer is shown in a ribbon representation (green and magenta) and retinal molecules are depicted by a stick model (pink). Blue lines represent the cell membrane.

the primary sequence identity between ChR and BR is as low as 15%, the overall structure of ChR is well superimposed on that of BR. TM3-6 are very similar, and the position of retinal is well conserved, whereas there are two distinct features between ChR and BR. First, ChR has an additional N-terminal and a C-terminal domain, and more importantly, the extracellular sides of TM1 and TM2 are tilted compared with those of BR. Because of this tilt, ChR has larger pores formed by TM1, 2, 3, and 7. The calculated electrostatic surface potential reveals that such a pore is strongly electronegative, so we assume that this pore act as the cation-conducting pathway in ChR (Fig. 2). To verify this hypothesis, we expressed the mutants of the pore-lining residues in HEK293 cells and recorded photocurrents in response to blue light pulses. Most mutants showed altered properties, including photoconductance, kinetics, and ion preference. Therefore, we suggest that this pore actually functions as the cation-conducting pathway.

While this putative cation-conducting pathway is opened toward the extracellular side, the cytoplasmic side of this pathway is occluded by Glu129 (Fig. 3). Although the calculated pKa of Glu129 suggests that this residue is protonated in the closed state, the E129Q mutant shows a strongly decreased photocurrent. Thus, we assume that Glu129 acts as the putative channel gate and the gating is regulated by the protonation change of Glu129 during the photocycle. The results of a recent FT-IR study also support our idea [4].

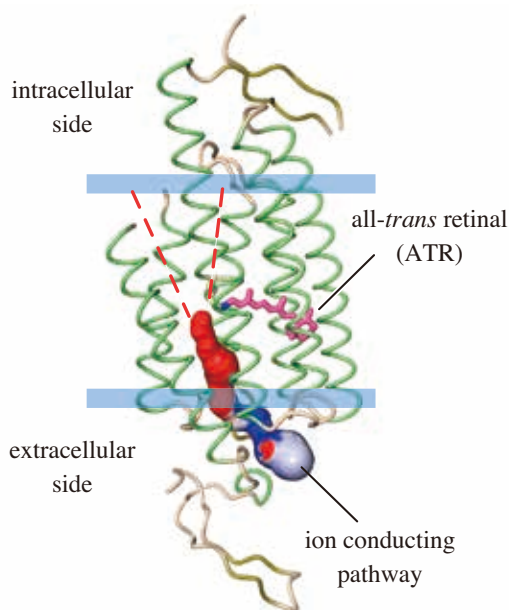


Fig. 2. Solvent accessible surface of the putative ion-conducting pathway. Blue and red indicate the positive and negative potentials, respectively. Putative intracellular vestibule is shown by red dashed line.

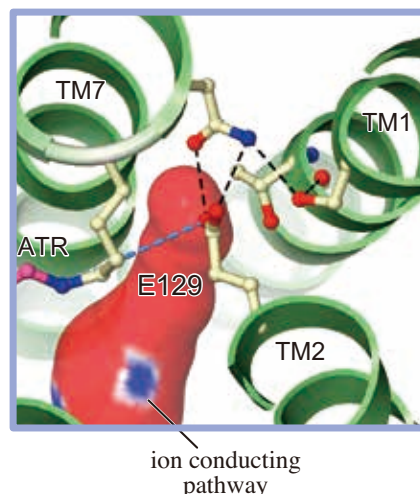


Fig. 3. Possible channel gate. Hydrogen bonds are shown as black dashed lines.

In the present study [5], we determined the first crystal structure of a light-gated cation channel, in the closed/dark state at 2.3 Å resolution, and provided insight into ChR dimerization, retinal binding, and cation conductance. In the field of ChR, there are two hypotheses about the cation-conducting pathway. One is that the pathway comprises helices from a single ChR molecule, and the other is that the ChR dimer assembles to form the conducting pathway using elements from each of the two ChRs. The results of this study strongly supports the former hypothesis and will accelerate the basic mechanistic understanding of this remarkable photoreceptor protein. This high-resolution information, along with electrophysiological analyses, will also guide the design of ChR variants with ideal properties.

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