Structure of Squid Rhodopsin

Rhodopsin is the primary molecule in the visual signaling cascade in vertebrate and invertebrate photoreceptor cells. Upon light absorption, the retinal chromophore in rhodopsin undergoes isomerization from the 11-*cis* to the all-*trans* configuration, which initiates the photoactivation process. In vertebrate retinas, the final photoproduct, meta-rhodopsin, activates a subtype of heterotrimeric G-protein, transducin (Gt), which in turn activates phosphodiesterase, an enzyme that degrades the second messenger, cGMP. In the retinas of many invertebrates such as insects and cephalopods, on the other hand, photoactivated acid meta-rhodopsin stimulates a Gq-type G-protein, and then the G-protein activates phospholipase C, an enzyme that hydrolyzes phosphatidyl inositol into the second messengers inositol trisphosphate and diacyl glycerol [1]. The same cascade is used by many G-protein-coupled hormone/neurotransmitter receptors such as serotonin and acetylcholine receptors, indicating that invertebrate rhodopsin is a prototypical member of the G-protein-coupled receptor (GPCR) family. The structural information of invertebrate rhodopsin would be useful to elucidate a common mechanism of Gq-type G-protein activation by GPCR.

We performed X-ray crystallographic analysis of invertebrate squid (*Todarodes pacificus*) rhodopsin. Squid rhodopsin contains 448 amino acids, i.e., 100 residues longer than those of vertebrate counterparts, mainly due to the unique C-terminal extension with repeated proline-rich sequences. After enzymatic deletion of the extended C-terminus, squid rhodopsin was extracted selectively from microvillar membrane with octylglucoside in the presence of zinc ion, and crystallized into the hexagonal *P62* crystal. We collected diffraction data from a frozen crystal of squid rhodopsin at beamline **BL38B1** and determined its crystal structure at 2.5 Å resolution [2].

The polypeptide of C-terminally truncated squid rhodopsin, which was traced from Glu 9 to Glu 358, is composed of seven transmembrane helices (from I to VII) and two cytoplasmic helices (VIII and IX) (Fig. 1). The overall structure revealed new features of Gq-coupled receptors. Helices V and VI extend into the cytoplasmic medium and, together with a hydrophilic helix IX, they form a highly organized structure of about 25 Å length from the membrane surface. The calculated map of electrostatic potential on the protein

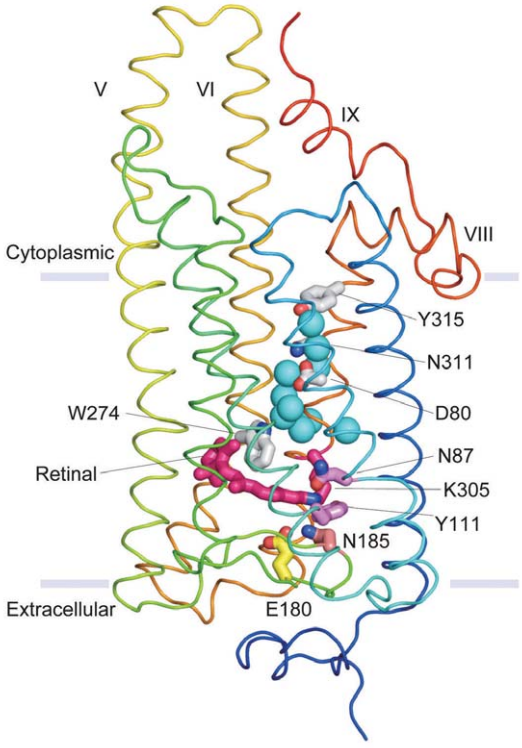

Fig. 1. Crystal structure of C-terminally truncated squid rhodopsin. Helices are color-coded from blue (helix I) to red (helix IX). Retinal-Lys305 chain (pink), water molecules in an interhelical cavity (cyan), and several key residues (yellow, orange, magenta, and white) are depicted.

surface shows that positively charged residues are crowded into the N-terminal end of helix IV and the second cytoplasmic (C2) loop, whereas negatively charged residues are clustered along helix IX. This structural motif and charge distribution in the cytoplasmic side seem to explain the selective interaction of squid rhodopsin with Gq-type G-protein.

Invertebrate rhodopsin is known to exhibit a bistable property; that is, its acid-meta state is thermally stable and reverts to the dark state upon absorption of a second photon, whereas vertebrate rhodopsin undergoes an irreversible photobleaching reaction. Reflecting this difference, squid rhodopsin has a unique feature in the retinal binding pocket, which is quite different from that of bovine rhodopsin [3]. The sole anionic residue Glu 180 in the retinal binding region is too far from the Schiff base to have a direct interaction; in fact, the side chain of Asn 185 is located between them. In the dark-state structure, the protonated Schiff base is stabilized by hydrogen bonds with either the side-chains of Asn 87 or Tyr 111. Because the OH group of Tyr 111 is kept neutral in both the dark state and the acid metarhodopsin state [4], it is suggested that in the acid-meta state, Asn 185 mediates an indirect interaction between Glu 180 and the protonated Schiff base. Thus, our structure provides a reasonable explanation for a bistable property of squid rhodopsin, in which the retinal chromophore is not released from its binding pocket after the photoactivation.

Squid rhodopsin possesses a large interhelical cavity that is filled by nine water molecules. Together with polar residues, these water molecules form a long hydrogen-bonding network, which extends from the retinal-binding pocket to the cytoplasmic surface. A recent FTIR study has shown that more than eight water molecules change their vibration frequencies upon formation of bathorhodopsin [5]. It is thus suggested that a light-induced conformational change propagates along the water cluster towards the cytoplasmic side during the photoactivation process. As the amino acid residues around the water cluster are highly conserved in GPCRs, the rearrangement of the water molecules in the interhelical region seems to play an important role in the activation process of G proteins.

In the $P6_2$ crystal, intramembrane dimerization is observed around a crystallographic twofold axis (Fig. 2). In this dimeric association, the protein-protein interactions are reinforced by phospholipids located at the intradimer interface. The crystal structure shows another type of protein-protein interaction that leads to intermembrane dimerization; i.e., the N-terminal polypeptides of neighboring monomers are tightly associated via salt bridges. It should be noted

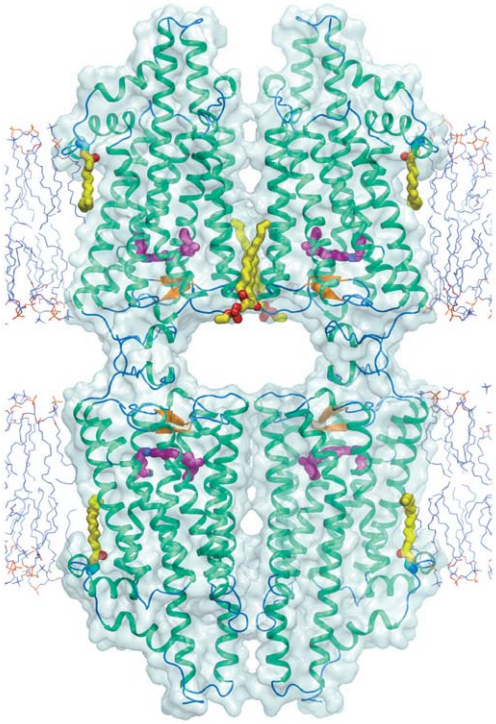

Fig. 2. A tetrameric structural model of squid rhodopsin incorporated into the lipid bilayer.

that two adjacent intramembrane dimers form a tetrameric structure around a noncrystallographic twofold axis. In this tetramer, four retinylidene chromophores are orientated nearly parallel with one another. It is possible that such tetramers are arranged in the apposed microvillar membranes so that the absorption dipole moments of all the retinal chromophores are aligned in parallel with the microvillar axis. As all the microvillar membranes in each photoreceptor cell are arranged in a particular direction, our crystal data provide a structural basis for the detection of polarized light by invertebrate eyes.

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