Crystal structure of squid rhodopsin at 2.5 Å resolution

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Like other visual pigments, invertebrate rhodopsin contains 11-cis retinal attached to a lysine residue, whose photoisomerization results in the activation of a heterotrimeric G-protein (guanine-nucleotide-binding-protein). In contrast to vertebrate vision, in which signal transduction is mediated by the second messenger cyclic GMP, invertebrate phototransduction utilizes an IP3 signalling cascade in which a Gq-type G-protein is stimulated by photoactivated rhodopsin.4 Since the latter is utilized by a large number of G-protein-coupled receptors (GPCRs), invertebrate rhodopsin is regarded as a prototypical member of GPCRs.

We have recently succeeded in crystallizing squid rhodopsin into a hexagonal $P6_3$ crystal [1]. This crystal diffracted X-rays up to 2.5 Å resolution. The structural analysis showed that membrane region of squid rhodopsin consists of seven α-helices (I–VII). Interestingly, helices V and VI extend into the cytoplasmic medium and, together with two cytoplasmic helices (VIII and IX), they form a rigid protrusion of a length about 25 Å from the membrane surface. This peculiar structure, which is not seen in bovine rhodopsin, seems to be crucial for the recognition of Gq-type G-protein. The retinal Schiff base is hydrogen-bonded to either Asn87 or Tyr111 and it interacts indirectly with the putative counterion Glu180 via the side chain of Asn185. Nine water molecules fill the interhelical cavity, forming a long hydrogen-bonding network which extends from the retinal-binding site to the putative G-protein-binding site on the cytoplasmic surface. Since residues encapsulating these water molecules are highly conserved among GPCRs, it is possible that the intraprotein water cluster has a striking functional role in the photoactivation processes of squid rhodopsin. In the crystal, tight association is formed between the N-terminal polypeptides of neighbouring monomers; this intermembrane dimerization may be responsible for the organization of hexagonally packed microvillar membranes in the photoreceptor rhabdom.

[1] Murakami, M., Kitahara, R., Gotoh, T. & Kouyama, T., Crystallization and crystal properties of squid rhodopsin (2007). *Acta Crystal.* **F63**, 475-479.