ATOMIC STRUCTURE OF BOVINE RHODOPSIN: A SEVEN TRANSMEMBRANE RECEPTOR

Seven transmembrane (7TM) receptor is a major protein family. The interest in 7TM receptor is growing not only due to its biological significance but also because of its practical importance in the post-genomic era. It could be a potential target for developing therapeutics for diverse ailments, such as general pain, allergy, circulation disorder, CNS (central neural system) disorder and AIDS. In vertebrates, the 7TM receptor family is believed to contain at least 600 members, wherein 370 odorant receptors have been identified in the human genome. 7TM receptors bind a variety of ligand classes: retinals, bio-organic amines, odorants, amino acids and their derivatives, peptides, proteins, nucleic acids and their derivatives, lipids and their derivatives, and glycoproteins. Many of these 7TM receptors are primary cellular acceptors of environmental stimuli for many processes, such as vision, odorant sensing, neuro-transmission, hormone regulations and chemotaxis.

In cellular signal transduction, the molecular mechanism of the 7TM receptor is commonly triggered via the activation of a limited number of GTP-biding proteins. For this reason, the 7TM receptor is often called as G-protein coupled receptor (GPCR). The molecular structure of the 7TM receptor is similar in its seven transmembrane helix bundle; furthermore its dominant sub-family, rhodopsin family members, shares highly conserved residues in amino-acid sequences. Thus, most of the 7TM receptors are thought to be activated via a common mechanism. To elucidate the molecular mechanism of the 7TM receptor, the experimental atomic model of a visual pigment, rhodopsin in the retina, recently has been determined using synchrotron X-ray crystallography at beamline **BL45XU** by the MAD method, while low-resolution models had been previously obtained using electron diffraction methods for frog or bovine rhodopsin [Schertler *et al.*].

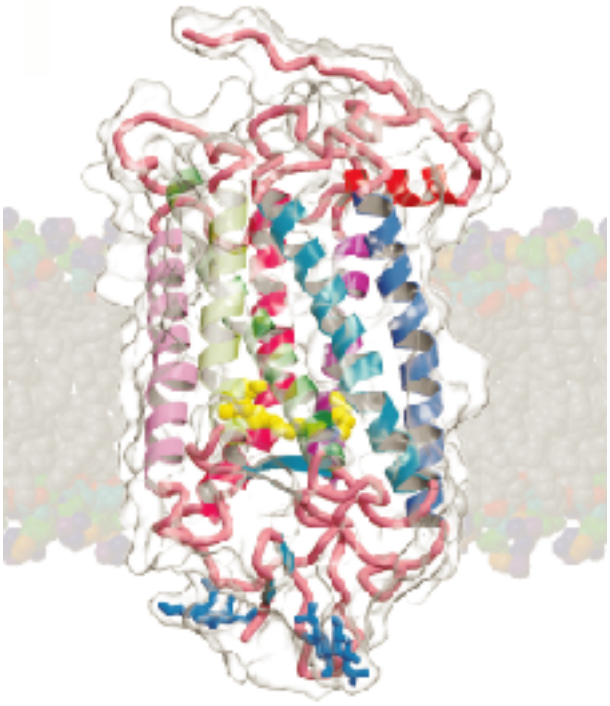

Fig. 1. Structure of bovine rhodopsin. Cartoon model of bovine rhodopsin structure with transparent molecular surface accompanied with putative lipid bi-layer model. Seven trans-membrane helices are colored in rainbow from α-helix I to VII with additional short helix VIII in red. Bound 11-cis-retinal shows ball model in yellow. Four short β-strands of N-terminal domain are in sky-blue. All loops are in pink wire with blue colored sugars.

The first structure of the 7TM receptor determined for bovine rhodopsin which preserved 11-*cis*-retinal, a vitamin A derivative, revealed several interesting features with the common 7TM bundle in an inactive state (Figs. 1 and 2) [1]. Additional amphiphilic short helix VIII is located directly adjacent to the 7th TM helix on the putative membrane surface (Fig. 3). The helices VII and VIII nearly form a right angle, supported by the aromatic ring stacking of the conservative Tyr^{306} and Phe^{313} (Fig. 4D) [2]. The N-terminal domain folds compactly in a two-layered structure (Fig. 3). The two layers are composed of an N-terminal region and the long loop between helices IV and V with an S-S bridge of the conserved Cys^{187} and Cys^{110} at the end of helix III (Fig. 4A).

Fig. 3. Stereo view of rhodopsin cartoon model.

Conversely, cytoplasmic loops almost open to form the putative interface with the G-protein, transducin [3]. Most of the highly conserved amino-acid residues among rhodopsin family members form non-covalent bonds to support the inactive form. The GPCR motif of Glu^{134}-Arg^{135}-Tyr^{136} interacts with Glu^{247} and Thr^{251} of helix VI at the cytoplasmic end of helix III (Fig. 4B). Asn^{55}, Asp^{83}, Asn^{78} and Trp^{161} are shown to constitute hydrogen-bond networks (Fig. 4C) [4]. Many parts of the seven transmembrane helices are irregular, due to many existing α-helix-destructive Gly and Pro residues

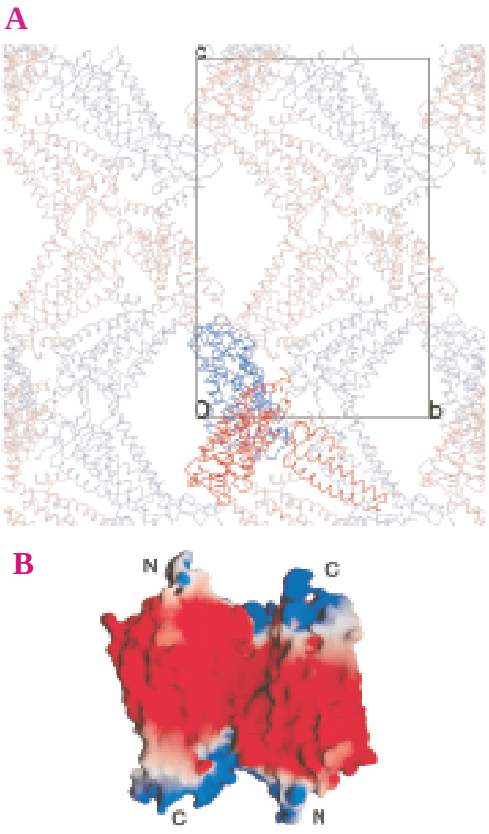

*Fig. 2. Crystal packing of bovine rhodopsin. **A)** Projection image of crystal packing onto **b**-**c** plane. Unit cell is indicated by square with the origin (**0**) and lattice (**b** and **c**). **B)** Dimer in a crystallographic asymmetric unit with water accessible surface colored by electrostatic potential, positive in blue and negative in red. Labels of **N** and **C** indicate the direction of **N**-and **C**-termini, respectively. Each molecule in the dimer packs in up side down artificially.*

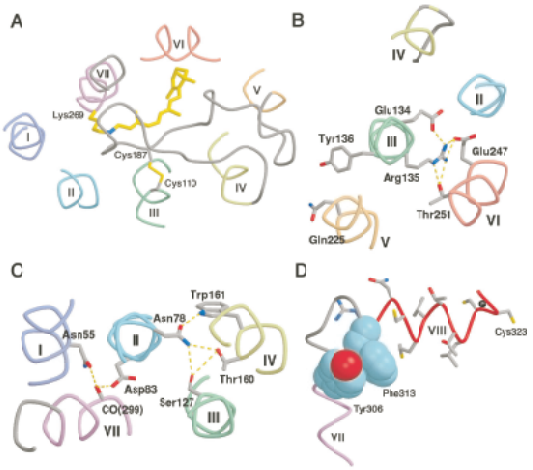

Fig. 4. Structure of highly conserved residues among 7TM receptors.

contained in the 7TM helices (Fig. 3). In fact, α-helices II, VI and VII are clearly kinked, and, in helix VII, several residues around Lys^{296} that binds 11-*cis*-retinal via the Schiff base form a 3_{10} helix. These bent-helices form a spacious cavity that is larger than which is needed to accommodate the bound retinal, while the cytoplasmic side of the helices is tightly bundled (Fig. 3 and Fig. 4A) [6]. The configuration of the reverse agonist, 11-*cis*-retinal, is bow-shaped in 6s-*cis*, 12s-*trans,* and *anti*-C=N of the Schiff base with a direct salt bridge of Asp^{113} (Fig. 5).

The atomic structure of inactive rhodopsin consistently reinforces experimental results obtained to this point, including visual diseases, yielding many clues for further study of the molecular mechanism of 7TM receptor activation, as well as the molecular basis of color vision. Further studies on the activated form of rhodopsin will elucidate novel features of the molecular basis of the 7TM receptor, while the structure of the inactive form is well-suited to the design of antagonists.

Masashi Miyano

SPring-8 / RIKEN

E-mail: miyano@spring8.or.jp

References

[1] J. Bockaert and J. P. Pin, EMBO J. **18** (1999) 1723.
[2] V. M. Unger *et al.*, Nature **389** (1997) 203.
[3] T. Okada *et al.*, J. Struct. Biol. **130** (2000) 73.
[4] Krzysztof Palczewski, Takashi Kumasaka, Tetsuya Hori, Craig A. Behnke, Hiroyuki Motoshima, Brian A. Fox, Isolde Le Trong, David C. Teller, Tetsuji Okada, Ronald E. Stenkamp, Masaki Yamamoto and Masashi Miyano, Science **289** (2000) 739.

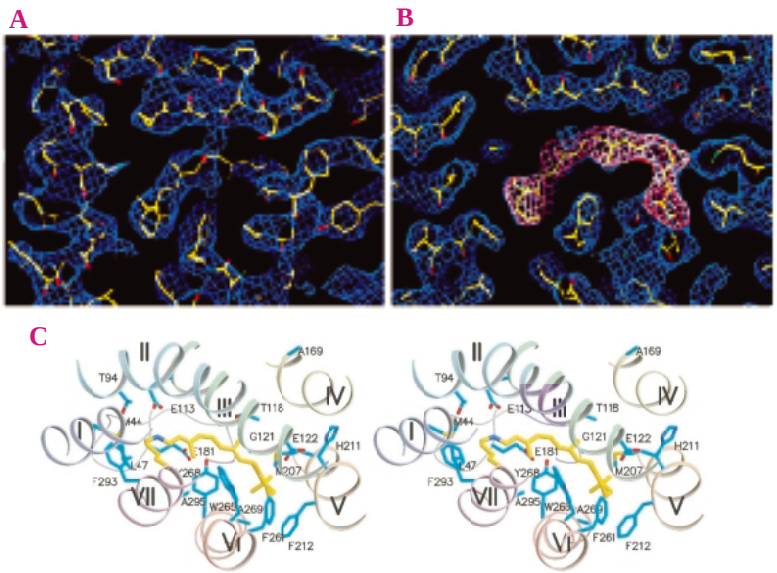

Fig. 5. Closed up structure of bound retinal vicinity.
***A)** Experimental electron density map with the current model at 3.3 Å.*
***B)** 2FoFc and omit FoFc map calculated using model phasing at 2.8 Å.*
***C)** Cartoon model of retinal vicinity in stereo.*