Crystal Structure Analyses of Bovine Rhodopsin

Tetsuji Okada 0003789, Kazuki Takeda 0003786, Hidenori Sato 0003787

Department of Physics, Graduate School of Science, Nagoya University, Japan

G protein-coupled receptors (GPCR) form a membrane protein superfamily that mediates the actions of extracellular signals, such as light, hormones and neurotransmitters. Although structural feature of this family has been established by amino acid sequences that have seven hydrophobic segments, a knowledge of their three-dimensional structure is an important step towards an understanding of signal transduction mechanisms and rational drug design.

Rhodopsin is a photoreceptor protein which is responsible for visual perception, and is a member of GPCR. Upon absorption of a photon, 11-cis-retinal chromophore isomerizes to all-trans form within hydrophobic region of the protein. This event leads to irreversible conformational change of rhodopsin, by which activation of many copies of G protein is achieved.

A low resolution structure of the seven transmembrane segments of rhodopsin has been obtained by electron cryo-microscopy on two-dimensional crystals. The effective resolution of the map is 7.5 Å in the plane of the membrane and 16.5 Åperpendicular to it (1). Although it has been proposed that the transmembrane structure can be utilized as a model for most of the GPCR (2), little informations are available for the binding site of G protein. Therefore, it is desirable to carry out high resolution structural analyses of rhodopsin by X-ray crystallography.

Recently we have succeeded to establish a simplified procedure for purification of rhodopsin from bovine rod outer segments (3), and several types of three-dimensional crystals are obtained using the purified sample. Preliminary experiments indicated that all of the rhodopsin crystals were broken with visible light, and that one of the crystals diffracted X-ray at least 14 Å resolution at room temperature under dim red light condition.

The experiment at BL41XU was carried out both at room temperature and at 100 K under dim red light condition. The wavelength of X-ray was 0.7 Å and the crystal-to-detector distance was 560 mm. One of the crystals diffracted to 10 Å resolution at 100 K. The probable crystal system and the unit cell dimensions were suggested to be orthorhombic, a=146.1 Å , b=251.2 Å , c=133.1 Å , respectively.

Since the diffraction pattern also indicated the ice formation, further improvement of cryo-protectant condition is needed. The unit cell of the crystal examined seems to be too large to use for high resolution analyses, future experiments are planned to examine another types of rhodopsin crystals.

(1) Unger, V. M., Hargrave, P. A., Baldwin, J. M., and Schertler, G. F. X. (1997) *Nature* **389**, 203-206.
(2) Baldwin, J. M., Schertler, G. F. X., and Unger, V. M. (1997) *J. Mol. Biol.* **272**, 144-164
(3) Okada, T., Takeda, K., and Kouyama, T. (1998) *Photochem. Photobiol.* **67**, 495-499.