X-ray Crystallographic Analyses of Bovine Rhodopsin and Its Early Photo-Intermediates

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The primary process in visual signal transduction is a photo-isomerization of 11-cis-retinal to the all-trans form in rhodopsin and color pigments. It is a common mechanism by which these retinal proteins are activated, subsequently leading to the active conformation that is capable of catalyzing nucleotide exchange reaction in hetero-trimeric G protein. Such a trigger by the chromophore corresponds to binding of a diffusible ligand in other G protein-coupled receptors. Thus, chromophore binding site in rhodopsin is a key to understand molecular mechanism of receptor activation.

The 11-cis-retinal chromophore is covalently bound to Lys296 in the middle of transmembrane (TM) helix VII via protonated Schiff base linkage, which is stabilized by a counterion Glu113 in helix III. The stabilization mechanism of this salt bridge has been a focus of many previous studies because it is well established that proton transfer from the Schiff base to the counterion is one of the determinants of rhodopsin activity.

In the TM hepta-helical bundle of archeal retinal proteins such as bR, hR and sRII, each of whose structure has been determined at atomic resolution, some water molecules was found along the direction parallel to the membrane normal and a part of it appeared to mediate interaction between the protonated Schiff base and the conterion. On the other hand, previous crystal structure models of bovine rhodopsin were not sufficient to define water distribution in the TM region.

The two data sets collected during this beamtime and another one from the previous run (2001A0275) were used to explore water molecules in the TM region of bovine rhodopsin. In the refined crystal structure model using x-ray diffractions up to 2.6 Å, we assigned seven water molecules for each of the two molecules in an asymmetric unit. Two of them were found to interact with the 11-cis-retinal protonated Schiff base indirectly, presumably providing a hydrogen-bond network for regulation of not only activity but also light absorption property.

We have also confirmed by the separate experiments that spectroscopic properties of rhodopsin in the three-dimensional crystals are similar to that has been observed in solution. Based on these results, further analysis are still in progress using a number of other data sets on the early photo-intermediates of bovine rhodopsin.

Elucidation of the regulatory mechanism based on the crystal structures of *rat* GTP-CH-I and GFRP

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In mammals, GTP cyclohydrolase I (GTP-CH-I; E.C. 3.5.4.16; 230 residues per monomer) is the first and rate-limiting enzyme in the biosynthesis of 6*R*-L-erythro-5,6,7,8-tetrahydrobiopterin (BH_4) from GTP. BH_4 is an essential cofactor for nitric oxide synthase and also for three enzymes, phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase, which play key roles in producing neurotransmitters such as catecholamines from phenylalanine and serotonin from tryptophan. Therefore, the end product BH_4 (and also BH_2) of the pterin pathway and the starting substrate phenylalanine of the catecolamine pathway are allosteric effectors for GTP-CH-I. Surprisingly, these effectors have no effect on the GTP-CH-I activity without its feedback regulatory protein (GFRP; 83 residues per monomer). Moreover, in the presence of these effectors GFRP is unable to bind GTP-CH-I. Intensive biochemical analyses have suggested that GTP-CH-I and GFRP can form a BH_4–induced inhibitory complex in the presence of the substrate GTP or its non-productive substrate analogue dGTP. GTP-CH-I and GFRP can also form a stimulatory complex in the presence of >200 μM L-phenylalanine, which is the substrate of phenylalanine hydroxylase. Recent studies using gel filteration suggest that free GFRP forms a pentamer and two GFRP pentamers bind the GTP-CH-I decamer, thereby assembling a stimulatory $(GTP\text{-}CH\text{-}I)_{10}$-$(GFRP)_{10}$ complex. To understand the mechanisms by which GFRP regulates GTP-CH-I, we have crystallized the inhibitory ternary complex. The monomeric GTP-CH-I and GFRP have molecular masses of about 257 kDa and 10.0 kDa, respectively. The molecular mass of a single complex is thus approximatry 360 kDa.

A native data set was collected from one crystal at 100 K on the beamline BL41XU of SPring-8 using MAR CCD detector system by the rotation method with 1.5° oscillations. Diffraction data were processed and reduced with DPS/MOSFLM and SCALA. The crystals were found to belong to the monoclinic space group $P2_1$ with unit cell parameters a = 122.3, b = 111.8, c = 130.6 Å, β = 98.1°. Assuming the presence of one complex in the asymmetric unit, the calculated value of crystal volume per protein mass (V_M) is 2.33 $Å^3$ Da^{-1}. This value corresponds to a solvent content of approximately 47.2%. The intensity data collection on the beamline BL41XU gave a set of intensity data at 2.8 Å resolution. Self-rotation function analyses of the data revealed a strong peak representing a non-crystallographic fivefold axis. Sturcture analysis by molecular replacement using a stimulatory $(GTP\text{-}CH\text{-}I)_{10}$-$(GFRP)_{10}$ complex are in progress.

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