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X-ray crystallographic analysis of photoisomerase and the activation mechanism of rhodopsin

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1 Introduction

The visual function of animals is based on two isomeric forms of retinal (vitamin A aldehyde), 11-*cis* and all-*trans*. Upon photon absorption, 11-*cis*-retinal embedded within the transmembrane core of the dim-light photoreceptor rhodopsin isomerizes to the all-*trans* form, which results in conformational changes at the cytoplasmic surface where heterotrimeric G protein activation occurs during the limited lifetime of the activated rhodopsin.

The lifetime corresponds to the release of all-*trans*-retinal from the protein moiety of rhodopsin, opsin, which needs to be regenerated with 11-*cis*-retinal. Among some suggested mechanisms for the conversion of all-*trans*- to 11-*cis*-retinal, light-sensitive proteins, such as RGR in humans and retinochrome in squids, have been recognized as photoisomerases that carry out this reaction.

Light-triggered interaction changes between the retinal ligand and the surrounding polypeptide in rhodopsin and photoisomerase are particularly interesting subjects for X-ray crystallographic analysis. These proteins exhibit substantial sequence homology and are supposed to share a common heptahelical transmembrane core structure; however, the potency of G protein activation is significantly different from one another. Our project involves high-resolution studies on the photoreaction intermediate states of vertebrate rhodopsin [1, 2], allosteric regulation of receptor activation, and structure determination of photoisomerase.

2 Experiment

R Rhodopsin crystals were prepared by following a previously reported procedure [3], with and without the inclusion of some candidate molecules that are supposed to exhibit allosteric activity (Dr. C.L. Makino, personal communication). Crystals of photoisomerases were obtained as clusters of thin needles. X-ray diffraction experiments were carried out at the beamline NW-12A using crystals frozen under dim-red light (> 660 nm).

3 Results and Discussion

Detailed analysis of the diffraction data sets collected for crystals containing rhodopsin and a candidate allosteric molecule showed that β-ionone, which was supposed to bind to opsin allosterically, occupied a surface region around the extracellular loop connecting the transmembrane helices VI and VII (Fig. 1) [4]. Although the polypeptide structure did not appear to be affected by β-ionone in the presence of an orthosteric inverse-agonist 11-*cis*-retinal, this site could be the secondary retinoid binding site on the opsin molecule. Interestingly, this site is close to one of the possible entry/egress gates for retinal [5, 6]. A recent crystallographic study on the muscarinic acetylcholine receptor reported an allosteric binding site at the extracellular surface region [7]. Further studies regarding opsin with and without candidate allosteric molecules are important to find out how the constitutive activity of photoreceptor proteins can be regulated by the binding of diffusible molecules.

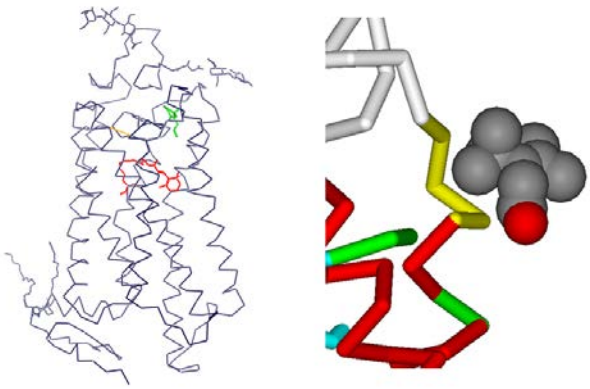

Fig. 1: β-ionone binding to bovine rhodopsin. Left, backbone structure with retinal bound to K296 (red) and β-ionone (green). Right, expanded view of β-ionone (space filling) and the binding site residues (yellow).

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References

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