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Review

Amino acid conservation and interactions in rhodopsin: Probing receptor activation by NMR spectroscopy☆

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ABSTRACT

Rhodopsin is a classical two-state G protein-coupled receptor (GPCR). In the dark, its 11-*cis* retinal chromophore serves as an inverse agonist to lock the receptor in an inactive state. Retinal–protein and protein–protein interactions have evolved to reduce the basal activity of the receptor in order to achieve low dark noise in the visual system. In contrast, absorption of light triggers rapid isomerization of the retinal, which drives the conversion of the receptor to a fully active conformation. Several specific protein–protein interactions have evolved that maintain the lifetime of the active state in order to increase the sensitivity of this receptor for dim-light vision in vertebrates. In this article, we review the molecular interactions that stabilize rhodopsin in the dark-state and describe the use of solid-state NMR spectroscopy for probing the structural changes that occur upon light-activation. Amino acid conservation provides a guide for those interactions that are common in the class A GPCRs as well as those that are unique to the visual system. This article is part of a Special Issue entitled: Retinal Proteins — You can teach an old dog new tricks.

1. Introduction

1.1. Rhodopsin as a model class A GPCR

The class A receptors are the largest of five distinct classes of G protein-coupled receptors (GPCRs). These receptors have a common 7 transmembrane (TM) helix architecture and catalyze the exchange of GTP for GDP in intracellular heterotrimeric G proteins when activated. One of the intriguing questions surrounding GPCRs is how the 7-TM helix structure has evolved to be capable of responding to a diversity of signals. Rhodopsin, the low-light receptor in vertebrates, has often been considered an exception within the class A GPCRs, but is now providing the basis for a common mechanism for activation.

Rhodopsin serves as a model GPCR. The receptor functions as an on-off switch where light energy is used to drive the protein from an inactive to an active conformation. All visual receptors from humans to squid contain the 11-*cis* isomer of retinal covalently bound within the 7-TM helix bundle (Fig. 1). In pharmacological terms, the 11-*cis* retinal chromophore acts as a potent inverse agonist when bound to the receptor and reduces basal activity of the apo-protein opsin to very low levels [1,2]. Specific molecular interactions, including those involving 11-*cis* retinal, have evolved to lock this light-activated receptor into an inactive conformation in the dark, allowing the reduction of thermal "noise". Upon light absorption, the retinal isomerizes within 200 fs [3], and then decays thermally through a series of spectrally distinct intermediates. The Metarhodopsin II (Meta II) intermediate corresponds to the active state of the receptor. Like rhodopsin, Meta II is stabilized by specific contacts that enable sufficient time for G protein activation. Differences in these helix–helix interactions are what distinguish the highly sensitive rhodopsin receptors that function in dim-light from the cone pigments that operate in ambient light conditions and require faster response and recovery times [4].

In the transition from Meta I to Meta II, the receptor undergoes a large conformational change. EPR studies revealed that there is an outward rotation of the cytoplasmic end of TM helix H6 in the transition to Meta II [5]. The motion of H6 opens up a cavity on the intracellular side of the receptor that serves as the G-protein binding pocket. The crystal structures of active opsin [6,7] showed that the outward rotation of H6 is accompanied by rotation of the intracellular portions of TM helices H5 and H7. Specific contacts between conserved tyrosines on these helices with Arg135 on helix H3 serve to stabilize H6 in an open conformation. The mechanism for how retinal isomerization is coupled to motion of helices H5, H6 and H7, however, is only now being unraveled.

1.2. NMR provides a complementary approach to X-ray crystallography

Rhodopsin was the first GPCR whose crystal structure was determined by high resolution [8]. The structure confirmed the seven-helix

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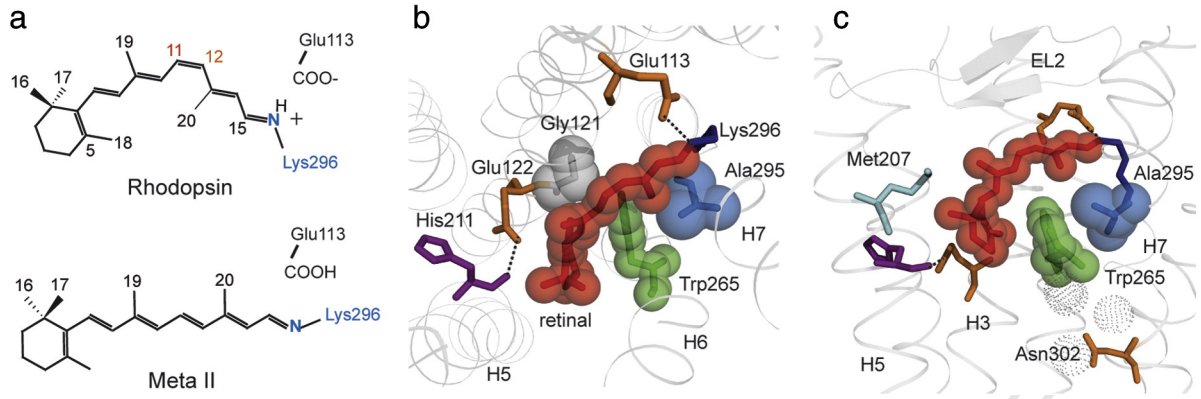

Fig. 1. Packing interactions of the retinal chromophore in rhodopsin. (a) Structures of the 11-*cis* retinal chromophore in rhodopsin and the all-*trans* retinal chromophore in the active intermediate, Meta II. (b, c) Two views of the structure of the retinal-binding pocket in rhodopsin (PDB ID: 1GZM). The view from the extracellular surface (b) shows several of the molecular interactions that lock the receptor in the inactive conformation in the dark. These include the Glu113–retinal PSB electrostatic interaction, the Glu122 – His211 hydrogen bonding interaction and the close van der Waals packing interaction between Trp265 and the retinal polyene chain. The view of the binding site from the membrane clearly shows how Trp265 is tightly packed between the retinal, Ala295 and Gly121. Ala295 is group-conserved across the class A GPCRs, while Gly121 is highly conserved within the opsin subfamily of receptors. The indole side chain of Trp265 is hydrogen bonded to Asn302 via water molecules (dotted spheres).

architecture and revealed the location of amino acids that are highly conserved across the large class A GPCR family. In the past 8 years a number of high-resolution crystal structures of class A GPCRs have been determined, mainly in their inactive forms. In addition to the visual pigments [6,7,9,10], high-resolution structures have been determined for amine [11–21], chemokine [22], mAChR [23,24], opioid [25–28], lipid [29] and δ-subfamilies of receptors [30], the latter including the olfactory receptors. The basic structural elements present in these structures are similar to those observed in rhodopsin. Comparison of rhodopsin with the ligand activated GPCRs shows that the largest structural diversity occurs in the N-terminus, the extracellular loops and the intracellular loops. On the extracellular side of rhodopsin, the second extracellular loop (EL2) is wedged between the TM helices and serves as a cap on the retinal-binding site. On the intracellular side, a short amphipathic helix is oriented roughly perpendicular to the seven TM helices.

In contrast, crystal structures of active GPCRs are fewer in number. Active state crystal structures of ligand-activated receptors that exhibit a large outward motion of H6 have been determined for the β2-adrenergic receptor with either a nanobody or the full length G protein bound to the intracellular surface [31,32]. In the presence of agonist alone, the structural changes in the ligand-activated GPCRs are more modest [15,33]. These receptors have relatively small barriers to activation in contrast to rhodopsin where light energy is needed to overcome the large thermal barrier to activation. In most GPCRs, multiple receptor conformations can be populated, which provides versatility in signaling and regulation [34].

Agonist-bound structures are available for the A_{2A} adenosine receptor [15,35], the β_1 adrenergic receptor (β_1AR) [33,36], the β_2 adrenergic receptor (β_2AR) [37] and the β_2AR in complex with Gs [32,38]. The largest change in these structures, as compared to the corresponding inactive conformation, is the displacement of TM helix H6. Agonist-induced conformational changes in other regions of these receptors appear to be minimal, which has left open the question as to how ligand binding triggers activation [39,40].

In order to correlate structure with function in GPCRs, a detailed understanding of the subtle differences between the various receptor conformations is required. High-resolution structural data are needed for each receptor state in as close to a native conformation as possible. Solid-state NMR spectroscopy has been particularly useful in characterizing the structure of dark rhodopsin and its intermediates. Deuterium NMR spectroscopy along with molecular dynamics has provided insights into the structure and dynamics of the retinal chromophore [41], while ^{13}C and ^{1}H NMR correlation spectroscopy has been used to probe the retinal structure and its interaction with surrounding amino acids [42,43] The use of selective pairs of ^{13}C labels has been useful for characterizing the conformation of the retinal [44] and internuclear $^{13}C\ldots^{13}C$ distances in the protein [45]. The receptor structure can be probed in a membrane environment using the native protein sequence or site directed mutants, and low temperature [46] provides a way to trap intermediates. In this review, we describe the use of solid-state NMR in the context of understanding the molecular interactions that stabilize the active and inactive states of rhodopsin. We highlight the retinal chromophore and its interactions with His211 on H5 and Trp265 on H6 to illustrate the types of structural information that can be obtained by NMR.

1.3. Molecular interactions and residue conservation provide insights into mechanism

Understanding the roles of the residues that are conserved across the class A GPCR family is an important component for developing a comprehensive description of the activation mechanism of rhodopsin. There are three levels of conservation that one must consider. The first level of conservation corresponds to the ~20 signature residues that have high sequence identity across the class A GPCRs. These residues are often grouped into structural and functional micro-domains that appear to mediate a common conformational switch involved in receptor activation. A second level of conservation corresponds to those residues that are highly conserved when considered as a group of similar amino acids. We have previously identified the group of small and weakly polar residues (Ala, Gly, Ser, Cys and Thr) as key determinants in helix–helix interactions [47,48]. However, there are other classifications of group-conserved amino acids, such as aromatic, charged or hydrophobic. The third level of conservation corresponds to those residues that are highly conserved within a receptor subfamily.

For example, in rhodopsin an aromatic cluster of amino acids is found on H6, which has relatively high sequence identity across the class A GPCRs. Trp265 lies within the arc formed by the retinal polyene chain and the Lys296 side-chain, and is packed between Gly121 and Ala295 (Fig. 1). Gly121 and Lys296 are highly conserved in the visual receptor subfamily and Ala295 is a group-conserved residue across the class A GPCRs. The 11-*cis* retinal appears to function as a clamp to prevent motion of Trp265 and H6 [49], thus locking the dark-state conformation. Isomerization of the retinal and motion of the β-ionone ring toward H5 appear to be essential for motion of Trp265 [49]. Together, the conserved amino acids form a network spanning all seven