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Review

Molecular simulations and solid-state NMR investigate dynamical structure in rhodopsin activation ☆

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ABSTRACT

Rhodopsin has served as the primary model for studying G protein-coupled receptors (GPCRs)—the largest group in the human genome, and consequently a primary target for pharmaceutical development. Understanding the functions and activation mechanisms of GPCRs has proven to be extraordinarily difficult, as they are part of a complex signaling cascade and reside within the cell membrane. Although X-ray crystallography has recently solved several GPCR structures that may resemble the activated conformation, the dynamics and mechanism of rhodopsin activation continue to remain elusive. Notably solid-state ^{2}H NMR spectroscopy provides key information pertinent to how local dynamics of the retinal ligand change during rhodopsin activation. When combined with molecular mechanics simulations of proteolipid membranes, a new paradigm for the rhodopsin activation process emerges. Experiment and simulation both suggest that retinal isomerization initiates the rhodopsin photocascade to yield not a single activated structure, but rather an ensemble of activated conformational states. This article is part of a Special Issue entitled: Membrane protein structure and function.

Contents

Abbreviations: CHARMM, Chemistry at Harvard Macromolecular Mechanics; EPR, electron paramagnetic resonance; FTIR, Fourier transform infrared; GPCR, G protein-coupled receptor; MD, molecular dynamics; Meta I, metarhodopsin I; Meta II, metarhodopsin II; 2MBD, 2-methyl-butadiene; 3MHT, 3-methyl-hexatriene; MM, molecular mechanics; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PSB, protonated Schiff base; RDC, residual dipolar coupling; RQC, residual quadrupolar coupling; SDPC, 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine; SDPE, 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine; QM, quantum mechanics

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1. Introduction

Rhodopsin is the G protein-coupled receptor (GPCR) responsible for dim light vision and plays a prominent role in our understanding of biological signaling. Investigating GPCRs lies at the forefront of pharmaceutical research, as they represent almost one-half of current drug targets [1]. In fact, rhodopsin is the most well-studied GPCR, on account of its relative ease of procurement and its well-characterized spectroscopic intermediates [2,3]. Upon photon absorption, an 11-*cis* → all-*trans* isomerization of the covalently bound inverse agonist retinal leads to initiation of the rhodopsin photocascade. This isomerization causes conformational changes within rhodopsin that allow interaction with the G protein transducin (G_t) in the cytosol. The signaling protein transducin acts as an intermediary between rhodopsin and its effector cGMP phosphodiesterase. Catalysis of GDP–GTP exchange by rhodopsin allows transducin to activate the cGMP phosphodiesterase, yielding hydrolysis of cGMP and closing of cyclic nucleotide-gated ion channels in the rod cellular plasma membrane. This last step leads to hyperpolarization of the rod and generation of a visual nerve impulse. Although the above signaling cascade is a blueprint for GPCR function, it remains incompletely understood at present—particularly for rhodopsin activation at the molecular level.

In this regard, structural biology has contributed insights into the workings of this system that are broadly significant for membrane function. The first GPCR crystal structure was of rhodopsin in the dark state [4]. Subsequent crystal structures of rhodopsin [5–10], opsin [11,12], the β_1- and β_2-adrenergic receptors [13–17], and the adenosine A_{2A} receptor [18] have now been solved, offering the potential for further mechanistic insight. Nonetheless, despite the availability of a number of GPCR structures, X-ray crystallography cannot completely explain membrane protein function. No structure has been solved in a native lipid bilayer environment. Additionally, each structure is a snapshot of the protein, and is unable to fully reveal the dynamics. Recently, our understanding of rhodopsin activation has been further advanced by reports of X-ray structures for the active Meta II state—yet, the results are complicated by opposite orientations for the retinal ligand obtained by different research groups [9,10]. Application of spectroscopic techniques such as nuclear magnetic resonance (NMR) are thus needed to provide structural data for trapped photointermediates in order to fill these essential gaps in knowledge of the activation process [19,20].

Below we summarize a solid-state ^{2}H NMR approach that is unique in that it can be used to investigate both structure and dynamics of retinal within the rhodopsin binding pocket [21]. The ^{2}H NMR method is mainly limited to the immediate vicinity of isotopic labels. However, computational simulations can add to the ^{2}H NMR data by examining the entire proteolipid system, thereby contributing to a more complete picture of the receptor activation process. Here we demonstrate how experiment and theory have a synergistic interplay that yields new insights into the activation mechanism of rhodopsin in a nativelike membrane environment. Solid-state NMR spectroscopy probes the changes in the local structure and dynamics of the retinal ligand of rhodopsin at a site-specific level. In addition, molecular dynamics simulations allow extension to longer time scales, thus enabling further investigation of the energy landscape of rhodopsin activation. Numerical simulations require accurate parameterization of the relevant molecular mechanics force fields, which has motivated us to carry out quantum mechanical calculations for retinal model compounds. Last, we show how the retinal methyl groups are implicated in rhodopsin activation, involving subsequent interaction with the signaling G protein transducin in visual excitation.

2. Rhodopsin: interface between experiment and simulation

Rhodopsin, the canonical GPCR, is composed of seven transmembrane helices connected by a series of extracellular and cytoplasmic loops [3]. Unlike most other GPCRs the activating ligand, retinal, is covalently bound to Lys^{296} on transmembrane helix 7 (H7). As noted above, upon absorption of a photon retinal undergoes an 11-*cis* → all-*trans* isomerization [2], initiating a photocascade culminating with activation of the G protein, transducin (Fig. 1). This photocascade consists of a spectroscopically well-defined series of intermediates: dark state → bathorhodopsin → lumirhodopsin → Meta I ⇄ Meta II [22]. It is important to appreciate that rhodopsin is unable to fully activate transducin until reaching the Meta II state. Because the equilibrium between the Meta I and Meta II states strongly depends on conditions such as pH, temperature, humidity, and membrane bilayer composition, one can physically trap these intermediates by

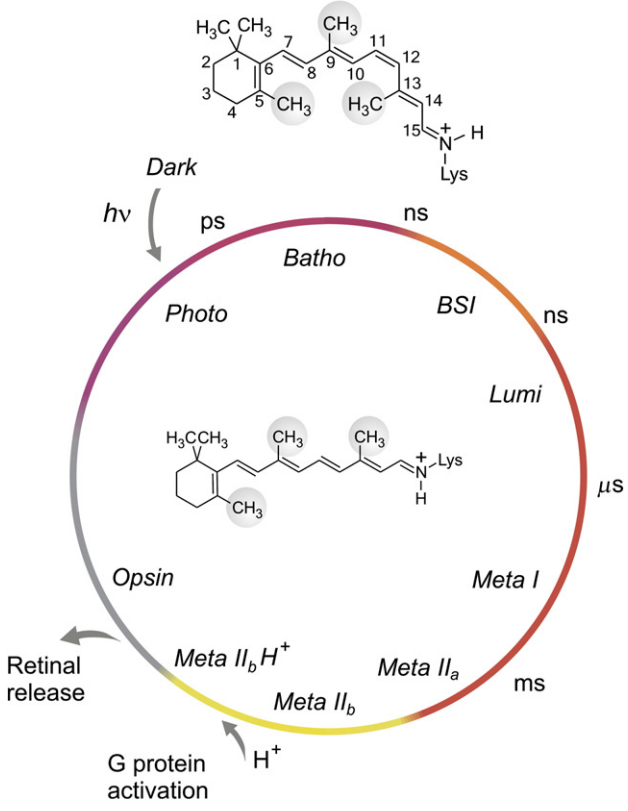

Fig. 1. Photocascade of rhodopsin underlies multiscale activation mechanism involving conformational changes with different time scales. Upon light absorption 11-*cis* to *trans* isomerization converts retinal from an inverse agonist to an agonist. Rhodopsin intermediates are designated as photorhodopsin, bathorhodopsin, blue-shifted intermediate (BSI), lumirhodopsin, metarhodopsin I, and metarhodopsin II. The Meta II state comprises an ensemble of Meta II$_a$, Meta II$_b$, and Meta II$_b$H$^+$ substates (activated ensemble mechanism). Note that various photoproducts can be trapped under different conditions of temperature, pH, or lipid composition.