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Retinal Dynamics Underlie Inverse-Agonist to Agonist Switch in Rhodopsin Activation

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Abstract

In seeking to understand G protein-coupled receptor (GPCR)-mediated signaling, X-ray and magnetic resonance approaches have played important roles—yet neither the protein dynamics nor the plasticity of GPCRs are amenable to study. Here we show that solid-state ²H NMR relaxation elucidates picosecond-nanosecond timescale motions of the retinal ligand that impact upon larger-scale functional dynamics of rhodopsin in membranes. A multiscale activation mechanism is put forward, whereby retinal initiates collective helix fluctuations in the Meta I–Meta II equilibrium on the microsecond-millisecond timescale.

TOC image

Rhodopsin is an important prototype for G protein-coupled receptors (GPCRs) that are implicated in biological signaling and constitute many human pharmaceutical targets. How the structure of the ligand is connected with larger-scale functional protein dynamics has remained elusive. Solid-state NMR relaxation shows that localized motions of retinal lead to collective fluctuations of transmembrane helices in the activation mechanism of the photoreceptor.

The molecular mechanisms of GPCR activation are the focus of considerable interest in cellular responses to biogenic amines and drugs, as well as taste, olfaction, vision, and a multitude of other biological signaling phenomena^{1,2}. Knowledge of the 3D structures of rhodopsin^{3,4} and the β_2 -adrenergic receptor¹ affords new opportunities for investigating GPCRs of human therapeutic significance. Despite the availability of crystal structures^{1,4}, understanding rhodopsin activation is challenging due to lack of atomistic-level data for the

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AUTHOR CONTRIBUTIONS

A.V.S. and M.F.B. designed the research. A.V.S. and G.F.J.S. performed the experiments. A.V.S., G.F.J.S., and K.M. M. analyzed the data. A.V.S. and M.F.B. wrote the paper.

signal transducing Meta II state². We applied solid-state ²H NMR methods⁵ to give a new axis of information—the fourth dimension of time—needed to more fully interpret structural studies^{4,6,7}. Our approach is quite different, as we investigate the intrinsic mobility of the ligand that changes during visual light excitation.

To explore the energy landscape⁸ for receptor activation, we measured ²H NMR relaxation times⁵ for the methyl (Me) groups⁹ of retinal bound to rhodopsin in the dark, Meta I, and activated Meta II states. Knocking out any of the Me groups yields back-shifting of the Meta I–Meta II equilibrium⁹, and loss of function. An overview of rhodopsin light activation^{10,11} illustrates the time scales of the major conformational transitions (Fig. 1a). Solid-state ²H NMR spectroscopy of dark-state rhodopsin with retinal ²H-labeled at the C5-, C9-, or C13-Me groups yields similar residual quadrupolar couplings (RQCs) (Figs. 1b–d). Yet pronounced variations are clearly evident in partially relaxed ²H NMR spectra (Figs. 1e,f), and from inversion-recovery curves (Fig. 1g) used to determine spin-lattice ($T_{1\rho}$) relaxation times.

A major strength of solid-state NMR spectroscopy is that membrane proteins are studied in a natural bilayer lipid environment¹². Because the retinylidene Me groups at the C5, C9, and C13 carbons (Fig. 1) strongly affect the Meta I–Meta II equilibrium⁹, we conducted ²H NMR relaxation measurements of these positions (see Supplementary Fig. 1).

Phosphoethanolamine (PE) head groups and unsaturated lipid acyl chains support rhodopsin activity¹³—they forward shift the Meta I–Meta II equilibrium, opposite to PC head groups. Hence we used a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayer to trap Meta I, and a POPC bilayer containing 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) to trap Meta II, as shown by UV-visible spectrophotometry (Supplementary Fig. 2). These results are explained by matching of the spontaneous (intrinsic) monolayer curvature (H_0) to the membrane bilayer; POPC with $H_0 \approx 0$ favors Meta I, whereas DOPE has $H_0 < 0$ and favors Meta II¹³. Such concepts have been previously reviewed¹³ and are receiving increased attention.

In the dark state, deformation of retinal^{12,14–16} (induced fit) gives striking differences in Me dynamics that are clearly evident in the ²H $T_{1\rho}$ relaxation times (Fig. 2). Arrhenius-type plots of the $T_{1\rho}$ data against inverse temperature for the retinylidene ligand in the dark, Meta I, and Meta II states are indeed unexpected: the $T_{1\rho}$ times for the C9- and C13-Me groups increase with temperature, whereas those for the C5-Me exhibit a distinct minimum (Fig. 2a). Briefly, the $T_{1\rho}$ minimum occurs at higher temperature (to the left) for slower motions (smaller pre-exponential factor and/or larger activation energy E_a), and conversely at lower temperature (to the right) for faster motions (larger pre-exponential factor and/or smaller E_a). At the $T_{1\rho}$ minimum, effective correlation times (τ_c) for Me spinning are related to the nuclear Larmor (resonance) frequency (ω_0) by $\tau_c \approx 1/\omega_0$, due to matching the power spectrum of the fluctuations to the nuclear spin energy gap. For the C5-Me group the correlation time for the motions is $\tau_c > 13$ ns below -100 °C. Interestingly, for the C9- and C13-Me groups the minimum falls outside the observed range (< -160 °C), so that $\tau_c < 1/\omega_0 \approx 13$ ns (see below).

The light-induced changes in the retinylidene dynamics are encapsulated by pronounced T_{1Z} differences in the Meta I and Meta II states (Figs. 2b,c). The short T_{1Z} relaxation times of the C5-Me group suggest the β -ionone ring maintains a predominantly 6-*s-cis* conformation¹², rather than 6-*s-trans* as in bacteriorhodopsin¹⁷. Notably¹⁸, the β -ionone ring is little affected by transitions among the dark, Meta I, and Meta II states—it retains nearly the same local environment¹². A shift of the T_{1Z} minimum for the C5-Me to lower temperatures in Meta II (Fig. 2c) implies a decrease in the local correlation time, *e.g.* due to lowering E_a by changing the C6–C7 torsional angle and/or increasing the ring mobility. For the functionally critical⁹ C9-Me group, a dramatic shift in T_{1Z} is seen in Meta I and Meta II that manifests an increase in E_a for Me spinning within the rhodopsin binding pocket. Similar E_a barriers of the C9- and C13-Me groups after 11-*cis* to all-*trans* isomerization are logical, as they are now on the same side of retinal (Figs. 2b,c).

Next, we applied NMR relaxation theory⁵ to analyze the molecular dynamics of retinal bound to rhodopsin. The T_{1Z} times were analyzed using either a 3-site jump model or a continuous diffusion model for rotation of the retinylidene Me groups⁵. Although the order parameters have little variation, large site-specific differences are evident in the pre-exponential factors and activation barriers (E_a) (Fig. 3a). For the C5-Me of the β -ionone ring ($E_a=10\text{--}15\text{ kJ mole}^{-1}$) the values exceed those of the other Me groups in dark, Meta I, and Meta II states. Surprisingly, the C5-Me barrier stays nearly unaltered up to Meta II, consistent with a predominantly 6-*s-cis* conformation of the β -ionone ring¹². Perhaps most striking, however, the C9-Me group—which is essential to rhodopsin function⁹—is literally a dynamical hotspot. Based on our quantum mechanical calculations (not shown), the remarkably small barrier to rotation of the C9-Me group ($E_a=2\text{ kJ mole}^{-1}$ in the dark state) (Fig. 3a) arises from non-bonded (1,6) interactions with hydrogen atoms H7 and H11 of the polyene chain (see Fig. 1a). By contrast, for the C13-Me group near the protonated Schiff base (PSB) the barrier is greater, due to non-bonded (1,7) interactions with hydrogen H10. Upon 11-*cis* to *trans* isomerization (Fig. 1a) (1,6) interactions of the C13-Me group occur with both polyene hydrogens H11 and H15, so the C13-Me barrier ($E_a=3\text{--}5\text{ kJ mole}^{-1}$) becomes comparable to the C9-Me barrier in the Meta I and Meta II states. Notably, in the sequence dark–Meta I–Meta II there is a progressive increase in E_a for the C9-Me of the polyene chain, accompanied by an opposite decrease for the C13-Me (Fig. 3a).

Rhodopsin (1U19)³ with retinal¹² inserted into the ligand binding cavity allows us to propose how local ps–ns motions detected by ²H NMR correspond to large-scale ms functional protein motions (Fig. 3b,c)^{6,10}. In the dark state, the low activation barrier of the C9-Me group signifies the absence of steric clashes, as it occupies a slot between Tyr²⁶⁸ and Thr¹¹⁸. In the Meta I NMR structure of retinal¹², the C9-Me acts effectively as a hinge point for retinal isomerization, causing the C13-Me and the C=NH⁺– groups to change orientation. Rotation of the C13-Me displaces the β_4 strand of the E2 loop towards the extracellular (*e*) side (Fig. 3c), disrupting a hydrogen-bonding network involving TM helices H4–H6, as suggested by a decrease in E_a for the C13-Me group. At the opposite end of retinal, the β -ionone ring is displaced towards the H3–H5 helical interface, giving a tighter packing for the C5-Me indicated by the relatively high E_a value in the Meta I state (Fig. 3a). The ionic lock involving the retinylidene PSB on H7 with its complex counterion due to Glu¹¹³ (H3) and Glu¹⁸¹ (E2) is broken by internal proton transfer from the PSB to Glu¹¹³ in

Meta II. Straightening of retinal causes the β -ionone ring to move away from Trp²⁶⁵ (H6) towards Glu¹²² (H3), disrupting a second hydrogen-bonding network connecting TM helices H3 and H5¹². Initial movement of helix H6 from the H1–H4 helical core is accompanied by displacement of helix H5, bringing Tyr²²³ closer to Arg¹³⁵ and Gly²³¹ near to Glu²⁴⁷ (Fig. 3d)¹⁹. Destabilization of the charge adduct of Glu¹³⁴ and Arg¹³⁵ of the E(D)RY sequence of helix H3 with Glu²⁴⁷ of helix H6¹⁹ yields transient exposure of transducin (G_t) recognition elements on the cytoplasmic (c) side (Fig. 3b). Receptor activation is further driven by protonation of Glu¹³⁴ from the aqueous medium¹¹.

According to the above picture, rhodopsin activation involves collective fluctuations of transmembrane helices H5 and H6 and the cytoplasmic loops in the Meta I–Meta II equilibrium. The reversible helix movements^{10,11} occur at kHz frequencies and match the catalytic turnover rate for G_t binding and activation by rhodopsin²⁰. Receptor activation is due to a fluctuating equilibrium among states and substates^{10,11}—an arresting illustration of the role of conformational entropy in GPCR biology. A key unanswered question is whether it will eventually be possible to quantitatively trap a unique activated rhodopsin conformation, or whether the receptor function is inextricably linked to dynamics of a conformational ensemble unlocked by photoisomerization of the retinal ligand.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Site-specific ^2H NMR relaxation illuminates functional dynamics of retinylidene methyl groups within binding pocket of rhodopsin. **(a)** Light absorption yields 11-*cis* to *trans* isomerization, converting retinal from an inverse agonist to an agonist by a series of intermediates with different time scales. **(b-c)** Solid-state ^2H NMR spectra for dark-state rhodopsin with 11-*cis* retinal deuterated at C5-, C9-, or C13- C^2H_3 groups in POPC bilayers (1:50 molar ratio). The ^2H NMR lineshapes indicate rapid axial spinning of C- C^2H_3 groups down to at least -160°C . **(e,f)** Partially relaxed ^2H NMR spectra for retinylidene C9- and C13- C^2H_3 groups of rhodopsin in aligned POPC membranes ($\theta=0^\circ$) at -150°C . **(g)** Inversion-recovery plots showing site-specific variations in spin-lattice (T_1) relaxation times for C9- and C13-Me groups at -150°C .

Figure 2.

Solid-state ^2H NMR of captures site-specific changes in retinal mobility during light activation of rhodopsin. Spin-lattice (T_{1z}) relaxation times ($\pm$ s.d.) of retinylidene methyl groups are shown *versus* reciprocal temperature in (a) the dark, (b) Meta I, and (c) Meta II states (-30 to -160 $^{\circ}\text{C}$). Methyl dynamics are described by an axial 3-fold jump model or a continuous diffusion model with coefficients $D_{\parallel}$ and $D_{\perp}$. In panels a–c rotation about the methyl 3-fold (C_3) axis corresponds to solid lines with $D_{\perp}=0$; the dashed lines include restricted off-axial diffusion ($D_{\perp}=D_{\parallel}$). Fits for the C5-Me in Meta I in panel b assume unlike rotational diffusion constants ($D_{\parallel} \neq D_{\perp}$) (dashed line), or two conformers with different bond orientations and axial diffusion coefficients (solid line).

Figure 3.

^2H NMR relaxation of retinal sheds new light on activation mechanism of rhodopsin. **(a)** Summary of analysis of solid-state ^2H NMR measurements. Order parameters of rapidly spinning methyl groups are designated by S_{C_3} ; the pre-exponential factor is k_0 for 3-fold axial jumps or D_0 for continuous diffusion; and E_a indicates the activation energy. (The diffusion model assumes either $D_{\perp}=0$ (right) or $\eta_D \equiv D_{\parallel}/D_{\perp}=1$ (left) except for the C5-Me in Meta I, where $\eta_D \approx 1$.) **(b–d)** Proposed activation mechanism for rhodopsin in membranes based on X-ray¹⁹, FTIR¹¹, and ^2H NMR data¹² (see text). Isomerization of retinal displaces the E2 loop towards the extracellular (e) side with fluctuations of TM helices H5 and H6 exposing transducin (G_t) recognition sites on the opposing cytoplasmic (c) surface. Figure produced (PDB code 1U19)³ using PyMOL [<http://pymol.sourceforge.net/>]