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Structural snapshots of conformational changes in a seven-helix membrane protein:

lessons from bacteriorhodopsin

Teruhisa Hirai¹, Sriram Subramaniam², and Janos K. Lanyi³

¹Three-dimensional Microscopy Research Team, RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan

²Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20817, USA

³Department of Physiology and Biophysics, University of California, Irvine, CA 92697, U.S.A

Abstract

Recent advances in crystallizing integral membrane proteins have led to atomic models for the structures of several seven-helix membrane proteins, including those in the G-protein coupled receptor family. Further steps towards exploring structure-function relationships will undoubtedly involve determination of the structural changes that occur during the various stages of receptor activation and de-activation. We expect that these efforts will bear many parallels to studies of conformational changes in bacteriorhodopsin, which still remains the best-studied seven-helix membrane protein (see Table 1 for a list of selected pdb depositions). Here, we provide a brief review of some of the lessons learned, the challenges faced, and the controversies over the last decade with determining conformational changes in bacteriorhodopsin. Our hope is that this analysis will be instructive for similar structural studies, especially of other seven-helix membrane proteins, in the coming decade.

Introduction

Bacteriorhodopsin and the related widely spread eubacterial and eucaryotic rhodopsins are seven-helix transmembrane proteins structurally similar to G-protein coupled receptors. Many of them are proton pumps, in which photoisomerization of the retinal from all-trans to 13-cis sets off a cyclic sequence of reactions that drive the transport of a proton across the cell membrane, while others have light-sensing function, analogous to bacterial chemotaxis receptors[1]. The numerous intermediate states in the reaction cycles, with transiently changed retinal and protein conformations, as well as changed protonation or hydrogen-bonding states of buried residues, have been studied extensively by spectroscopy [2,3], site-specific mutagenesis [4,5], and by comparisons across many species [6]. Together, these studies have established the connections between the spectroscopically observed intermediates BR-K-L-M-

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e-mail addresses: Teruhisa Hirai thirai@spring8.or.jp, Sriram Subramaniam ss1@nih.gov, Janos Lanyi jkllanyi@uci.edu.

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Conflict of interest statement

The authors declare no conflict of interest.

N-O-BR, and the functional aspects of the trajectory of the proton translocation from the retinal Schiff base to Asp-85 (coincident with formation of the M state), followed by reprotonation of the Schiff base by Asp-96 (coincident with formation of the N intermediate).

The spectroscopic changes observed in the visual rhodopsins of higher organisms triggered by light-activated retinal isomerization also involve changes in protonation of the Schiff base formed between retinal and the lysine residue in the seventh transmembrane helix [7]. The analogy between bacteriorhodopsin, rhodopsin and G-protein coupled receptors is even more compelling if one considers that rotation of the C13-C14 or C11-C12 retinal bond is an intense, local perturbation that spreads in discrete steps to the rest of the protein and drives functionally relevant conformational changes [8], much like ligand binding does in the receptors. It thus seems inevitable to speculate that understanding signal generation by G-protein coupled receptors will come from approaches similar to those employed already for the best-studied of the rhodopsins, bacteriorhodopsin. Here, we first present the structural foundations for the similarities in architecture between bacteriorhodopsin and the GPCR family, and follow this with an overview of the major findings and controversies encountered with structural studies of conformational changes in bacteriorhodopsin.

Structural similarities in the seven-helix family of membrane proteins

When aligned and superimposed, the known structures of the archaeal rhodopsin family (bacteriorhodopsin[9], halorhodopsin[10], sensory rhodopsin II[11], and xanthorhodopsin [12]) and the GPCR family (bovine[13,14] and squid rhodopsins[15], β_2 -adrenergic receptor [16], β_1 -adrenergic receptor[17], and A_{2A} -adenosine receptor[18]), respectively show diversity of tilt at the extracellular side of helix A (TM1), but each family shares similar folding patterns in the transmembrane region (Figs. 1a and b). Distinct differences between the GPCR and the archaeal families are also evident in the ligand binding region (loop 4-5 on the extracellular side), at loop 5-6, and in the existence of a conserved helix, H8, in the cytoplasmic side of GPCRs, where the G protein approaches. When the core part of the trans-membrane regions of bacteriorhodopsin and rhodopsin are aligned and superposed (Fig. 1c), the relative positions of helix C (TM3) and helix D (TM4) are displaced relative to each other, but the other parts are well aligned, including helix F (TM6), where the greatest large-scale movement after photoexcitation of both bacteriorhodopsin and rhodopsin occurs. The cytoplasmic end of helix F (TM 6) of bacteriorhodopsin and rhodopsin (opsin) shifts outward, with a pivot point at the equivalent position (Pro186 of bacteriorhodopsin and Trp265 of rhodopsin respectively)[19, 20]. The strong similarities in the folds and even in part of the conformational changes between these two protein families further underscores the likely relevance of lessons learned from bacteriorhodopsin for structural studies of GPCR conformational changes.

Large-scale conformational changes by diffraction methods

The existence of significant light-driven protein conformational change in bacteriorhodopsin was first reported from analyses of projection maps at ~ 7 Å resolution, using neutron and x-ray diffraction [21,22] and electron crystallography at ~ 3.5 Å resolution [23-25]. From more detailed structural investigation of bacteriorhodopsin trapped at various stages after illumination, Subramaniam et al. concluded that within ~ 1 msec after illumination a single, large protein conformational change (“opening”) occurs, which is eventually reversed upon thermal regeneration of the starting conformation. These initial studies were carried out using 2D crystals to obtain projection maps, and were extended subsequently to obtain a 3D structure of a bacteriorhodopsin mutant trapped in the open state, using cryoelectron microscopy and electron crystallography, at 3.2 Å resolution [19]. The most significant changes in conformation are at the cytoplasmic end of helix F, corresponding to an outward tilt pivoting around Pro 186, and a displacement of helix G.

Studies analyzing the stepwise large-scale changes in conformation were carried out using also x-ray crystallography of illuminated 3D crystals. These investigations led to a number of atomic models describing this conformational change [26-37]. A detailed comparison of the similarities and differences between x-ray and electron crystallographic studies led to two striking conclusions [38]. First, none of the X-ray crystallographic studies with illuminated 3D crystals captured the large conformational change identified in the electron crystallographic studies with 2D crystals. The closest connection between the electron and x-ray studies is the light-induced structural changes reported for the M intermediate state of the D96N mutant [39], where the residues in helices F and G displaying the largest movement in the electron crystallographic studies were found to become disordered upon illumination of the 3D crystals. The current consensus in the field is that the contacts between neighboring molecules in the 3D crystals inhibit the protein conformational changes that are observed in the 2D crystals, and that the best chance to observe functionally relevant structural changes may be to crystallize proteins already in this state.

The second striking aspect of the x-ray studies is that there are large differences in the coordinates reported by different groups for the same photocycle intermediates. This lack of consensus in the nature and extent of the large conformational change extends also to the small-scale changes in conformation in the vicinity of the retinal-binding site (see below). Further, the different structures reported for the same intermediate cannot be reconciled in terms of differing extents of change on a single conformational trajectory (Fig. 2a and its legend) or differences in composition of spectroscopic intermediates (Figs. 2c and d), leaving the sobering implication that the interpretation of structural changes by x-ray crystallography has been perhaps not as rigorous as it should have been [38].

Large-scale conformational changes by site-directed spin-labels

EPR spectra of site-directed spin-labels reveal changes in local mobility and side-chain distance upon conformational alterations of proteins [40], in the native membrane. Measurements of the former as transients at ambient temperature [41] and the latter in photostationary states at cryogenic temperatures [42,43] have confirmed the tilts of the cytoplasmic ends of helices F and G detected by electron crystallography (see above). There is disagreement, however, over when these changes occur, and what their magnitudes are. The results at some labeled positions indicate that the tilt of helix F begins to occur after formation of the M state [44], related possibly to two M substates [45], while at other locations it is delayed until decay of these M states [44]. Recent data suggest [46] that the timing of conformational change is more nuanced than previously thought: it reaches maximal amplitude when the buried proton donor Asp-96 becomes anionic, and begins to reverse as the aspartate is reprotonated from the cytoplasmic surface.

Although valuable as independent information, ESR spectroscopy has not resolved some of the issues in the diffraction studies. From spin-labels at various locations, the amplitude of the tilt of helix F has been estimated from 1 Å or less to as much as 5-6 Å [42,46,47]. The discrepancies may originate from genuine local differences, from unexpected rotamer conformations of the spin-labels at the membrane surface, or from uncertainties in the complex analysis of the spectral changes produced the spin-spin quenching.

Local conformational changes by x-ray diffraction

While one might have anticipated problems of crystal packing that would prevent large-scale conformational changes, and could even provide an explanation for the reported discrepancies among the different groups, there has been greater hope for consensus in the analyses of the small-scale changes that occur earlier in the photocycle. Unfortunately, this has not been borne out in the published findings. The earlier intermediates have generally been trapped at low

temperatures (~100K), after creating them in photostationary states at various temperatures [9,26-37,39,48,49]. Although this strategy does not produce genuine transient states, the structural information is usually regarded as relevant if the changes appear sequential and make some sense. However, there are numerous technical problems [50]. A significant source of error is the low information content of the data. Even the unusually good 1.4-1.6 Å resolution of the bacteriorhodopsin crystals from one of the groups [9,34], is barely sufficient to define these small local conformational changes. In fact, little or no evidence of such changes is found when high-resolution data is truncated to 2 Å [9]. A second source of error is that illumination produces mixtures of states, as the composition of the trapped photostationary state depends on the extinction coefficients of both the intermediates and the initial state at the chosen illumination wavelength, and the quantum yields of their interconversions. In practice, it was found that only the M intermediate could be trapped with high occupancy [26,39], while other states accumulated with occupancies as low as 20% in some cases [35].

Given these concerns, it is necessary to validate the data before calculation of models for the intermediate and the unchanged initial state. Illuminated vs. unilluminated difference F_{obs} maps (e.g., Royant et al. 2000, Sass et al. 2000, Lanyi and Schobert, 2007[27,28,34]) have not been definitive because of strong spatial overlap of the two formations and variations in the temperature factors of atoms. Comparing illuminated vs. unilluminated F_{obs} or $2F_{\text{obs}} - F_{\text{calc}}$ maps better reveals the locations of atomic displacements [9], and even more so when extrapolated to 100% occupancy [34]. One way to obtain a more reliable answer about conformational changes is to test the reproducibility of the models from multiple bacteriorhodopsin crystals at similar resolutions [34], but this has been done only rarely.

Description of the conformational changes at and around the retinal is crucial for mechanistic understanding of how photoisomerization drives transport. Two of the three reports on the first, the K intermediate, agree that the retinal is a twisted 13-cis isomer [9,29]. There is no consensus over the structure of the L state. In one report there are extensive atomic displacements in the protein as well as in the retinal, most prominently in a bend of helix C at Asp-85 toward the protonated retinal Schiff base [28]. A subsequent report from the same group contained a structure for L, from molecular dynamics, with significant alteration of the distance between the Schiff base and Asp-85 [37]. In another report, helix C was reported to be unchanged, but a key water molecule (402) was relocated from its original position between the Schiff base and Asp-85 to the cytoplasmic side of the retinal, where it was hydrogen-bonded to the now cytoplasmically oriented N-H bond of the Schiff base [35]. A water molecule in L near this location had been suggested also from observations of an O-H stretch frequency [51], but questions about assignment of the FTIR bands and the low occupancy of the intermediate raise doubts about this model. A third model [32,34], supported by replicate statistics, reported that the structural changes in L are mostly restricted to the retinal, with minor displacements of the indole rings of Trp-86 and Trp-182 that flank the retinal polyene chain [34]. Thus, there is extraordinary divergence in the conclusions reached by different analyses of the early intermediates of the photocycle.

Conclusions

What can we learn from the studies with bacteriorhodopsin? Perhaps the most important lesson is that initiating structural changes by ligand binding (or by light) in a 3D crystal may not be the best strategy, particularly when the changes are large-scale. Crystallizing receptors that already are in the desired conformations is likely to be much more successful, as already demonstrated elegantly in a recent crystallographic study of bovine opsin [20]. This approach potentially eliminates two of the greatest challenges with bacteriorhodopsin: poor occupancy of intermediates, and inhibition of structural changes in the matrix of a 3D crystal. Some of the other reasons for the discrepancies in the bacteriorhodopsin structures have to do with

variations in sample preparation protocols, lack of independent phasing (which could have reduced errors arising from molecular replacement), the exclusive use of spectroscopic markers to define protein conformational states that relies on the premise that the two are always in synchrony, and when confronted with inadequate crystallographic resolution, a willingness, perhaps, to over-interpret the observed structural changes.

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Figure 1.

Structural similarity among proteins in the seven-helix family. (a) Structural diversity of bacterial rhodopsins. Bacteriorhodopsin (PDB code; 1M0L) [9], halorhodopsin (1E12) [10], sensory rhodopsin II (1JGJ) [11], and xanthorhodopsin (3DDL) [12] are aligned using residues 11-29, 43-59, 82-98, 110-124, 137-153, 170-189, and 205-223 of bacteriorhodopsin and equivalent residues of others and shown colored in blue, light green, yellow, and coral respectively. (b) Structural diversity of the G-protein coupled receptor family. Bovine rhodopsin (PDB code; 1U19) [13,14], squid rhodopsin (2Z73) [15], β_2 -adrenergic receptor (2RH1) [16], β_1 -adrenergic receptor (2VT4) [17], and A_{2A} adenosine receptor (3EML) [18] are aligned using residues 37-62, 72-96, 109-137, 151-171, 201-223, 249-275, and 289-319 of bovine rhodopsin and equivalent residues of others and shown colored in green, light blue,

yellow, coral, and cyan respectively. (c) Direct structural comparisons of transmembrane regions of bacteriorhodopsin and bovine rhodopsin, as viewed from the cytoplasmic side. Residues 13-29, 43-59, 82-98, 110-124, 142-152, 170-189, and 205-222 of bacteriorhodopsin and residues of 44-60, 73-89, 117-133, 156-170, 213-223, 249-268, and 292-307 of bovine rhodopsin are aligned and shown colored in magenta and green respectively in stereo.

Figure 2.

Structural differences between different models reported for the M intermediate of the bacteriorhodopsin photocycle. (a) Plot of the root mean square deviation of C α atoms from the unilluminated state of bacteriorhodopsin in models of the M intermediate obtained using electron crystallography (blue; [19]), and with a model derived from X-ray crystallography that shows the largest changes (red; [27]). The inset shows an expanded view of the deviations at the cytoplasmic end of helix F for two M intermediate states shown in the main panel (blue and red, respectively) and from other reports of M intermediate structures (yellow; [39], purple; [26], green; [30], cyan; [36]). (b) Experimentally derived difference map (calculated using both amplitude and phase data from diffraction and image of triple mutant and wild type [38]) showing the nature and extent of the large conformational changes in the bacteriorhodopsin photocycle. (c and d) Difference maps computed from deposited coordinates for the M intermediate by electron crystallography [19] showing a close match (panel c) or by X-ray crystallography [27], showing a poor match (panel d) with the experimentally derived map in panel b. See [38] for a detailed discussion of the use of experimentally derived difference maps as a strategy for validating structures derived by crystallography for the conformational change.

Table 1
Selected non-illuminated and intermediate state structures of bacteriorhodopsin

PDB code (chain or model ID)	Sample	Crystallization method	State, illumination, pH condition	Occupancy (%)	Resolution (Å)	Residues in the model	Reference
1M0L	WT	lipidic cubic phase	Non-illuminated		1.47	5-156, 162-231	Schobert <i>et al.</i> , 2002[9]
1QKP	WT	lipidic cubic phase	K, 110K green	35	2.1	5-232	Edman <i>et al.</i> , 1999[48]
1M0K(2)	WT	lipidic cubic phase	K, 100K green	40	1.43	5-156, 162-231	Schobert <i>et al.</i> , 2002[9]
1IXF	WT	membrane fusion	K, X-ray	13	2.6	5-231	Matsui <i>et al.</i> , 2002[29]
1E0P(B)	WT	lipidic cubic phase	L, 170K green	70	2.1	5-232	Royant <i>et al.</i> , 2000[28]
1O0A (1)	WT	lipidic cubic phase	L, 170K red	~ 60	1.62	5-156, 162-231	Lanyi & Schobert, 2003 [32]
1UCQ	WT	membrane fusion	L, 160K green, 100K red	20	2.4	5-231	Kouyama <i>et al.</i> , 2004[35]
1VJM(2)	WT	lipidic cubic phase	L, 150K red	50	2.3	5-232	Edman <i>et al.</i> , 2004[37]
1KG8	WT	lipidic cubic phase	M ₁ , 230K yellow	100	2	5-155, 167-231	Facciotti <i>et al.</i> , 2001[49]
1M0M(2)	WT	lipidic cubic phase	M ₁ , 210K red or yellow	60	1.43	5-156, 162-231	Lanyi & Schobert, 2002 [31]
1P8H(1)	WT	lipidic cubic phase	M ₁ , 295K yellow	42	1.52	5-156, 162-231	Schobert <i>et al.</i> , 2003[30]
1FBK	D96G/F171C/F219L	2D crystal/EM	Model for M		3.2	4-228	Subramaniam & Henderson, 2000[19]
1CWQ(B)	WT	lipidic cubic phase	M, RT green	35	2.25	2-239	Sass <i>et al.</i> , 2000 [27]
1IW9	WT	membrane fusion	M, X-ray	~ 70	2.5	5-231	Takeda <i>et al.</i> , 2004[36]
1F4Z	E204Q	lipidic cubic phase	M ₂ , RT yellow	>93	1.8	5-156, 162-231	Luecke <i>et al.</i> , 2000[26]
1C8S	D96N	lipidic cubic phase	M _N , 290K yellow	100	2	5-153, 176-222	Luecke <i>et al.</i> , 1999[39]

PDB code (chain or model ID)	Sample	Crystallization method	State, illumination, pH condition	Occupancy (%)	Resolution (Å)	Residues in the model	Reference
1P8U(1)	V49A	lipidic cubic phase	N ⁺ , 295K red	37	1.62	5-156, 162-231	Schobert <i>et al</i> , 2003[30]
1JV7	D85S	lipidic cubic phase	Model for O		2.25	9-63, 78-232	Rouhani <i>et al</i> , 2001[52]
1X0I	WT	membrane fusion	acid blue state,model for O		2.3	8-233	Okumura <i>et al</i> , 2005[53]
RT, room temperature							