

Crystal Structure of Rhodopsin: A G-Protein-Coupled Receptor

R. E. Stenkamp,^{*[a]} D. C. Teller,^[b] and K. Palczewski^[c]

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1. Introduction

G-protein-coupled receptors (GPCRs) provide a molecular link between extracellular signals and intracellular processes. In the visual system, GPCRs such as rhodopsin and cone visual pigments absorb photons and initiate G-protein signal transduction processes that result in electrical signals processed by the brain.^[1–4] Rod cells, containing rhodopsin in their outer segments, respond to dim levels of light.^[3–5] Cone receptors contain cone opsins and respond to photons of different wavelengths, thus providing a basis for color vision.^[6]

All of these receptors contain a retinylidene chromophore that undergoes a *cis*–*trans* isomerization upon photon absorption. This conformational change leads to an altered structure at the protein's surface capable of binding transducin. This activates the G protein (transducin) which in turn activates a phosphodiesterase. The phosphodiesterase converts cyclic guanosine monophosphate (c-GMP) into GMP subsequently causing c-GMP-gated ion channels to close. The cell then hyperpolarizes and generates a signal that is processed by the retinal secondary neurons. Ultimately, the signal is transmitted to the brain.^[4, 7, 8]

The fundamental importance and natural abundance of rhodopsin has made it a prime candidate for biophysical studies. Cryo-electron microscopy (cryo-EM) of two-dimensional rhodopsin crystals provided the first views of the seven transmembrane helices forming the core of the protein's structure.^[9, 10] Other biophysical techniques, including NMR spectroscopy, have been applied to characterize the structure and function of the chromophore or the peptide fragments.^[1, 4, 11–21]

2. Crystallographic Structure Determination

The three-dimensional crystal structure of bovine rhodopsin was solved in 2000.^[22] Postdoctoral fellow Tetsuji Okada obtained crystals^[23, 24] by using protein generated from highly purified bovine rod outer segments by solubilization and centrifugation.^[24] Crystals were grown with vapor-diffusion methods with ammonium sulfate as the precipitant. The protein solution contained 80 mM Zn²⁺ ions to stabilize the protein, nonyl- β -glucoside as a detergent, and heptane-triol as an additive. Two properties of the crystals complicated the structure analysis: they were sensitive to white light and they were merohedrally twinned. All experiments were carried out under red light. The

twinning problem was alleviated by focusing on crystals with low twinning fractions for the initial structure solution. The structure was solved by using multiwavelength anomalous dispersion phasing methods for a mercury derivative of the natural protein.^[22] The structure has been refined at 2.8 Å resolution.^[25]

3. Structure

A stereoview of molecule A of ground state rhodopsin (Protein Data Bank entry 1HZX) is shown in Figure 1. The N terminus of the protein is located on the intradiscal, or extracellular, side of the membrane. Two oligosaccharide sites are located at residues 2 and 15. The main glycosylation pattern found in rhodopsin at these sites is (Man)₃(GluN)₁₀. Several of the carbohydrate residues have been added to the model on the basis of electron density maps.

The polypeptide passes through the membrane in seven helical segments labeled I–VII. The helices are irregular in length and orientation. Helix III is the longest and passes through the center of the protein. Helix VIII is a short helical segment on the cytoplasmic side of the membrane oriented with its helical axis parallel to the membrane surface. Two short β strands are located on the extracellular side of the protein near the retinal binding site (see below).

[a] Prof. R. E. Stenkamp
Box 357420
Department of Biological Structure
University of Washington
Seattle, WA 98195-7420 (USA)
Fax: (+1) 206-543-1524
E-mail: stenkamp@u.washington.edu

[b] Prof. D. C. Teller
Box 357350
Department of Biochemistry
University of Washington
Seattle, WA 98195-7350 (USA)

[c] Prof. K. Palczewski
Box 356485
Department of Ophthalmology
University of Washington
Seattle, WA 98195-6485 (USA)

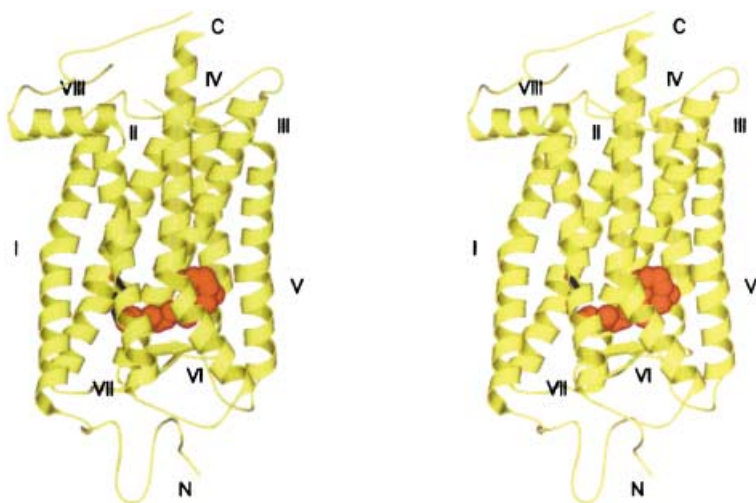


Figure 1. Stereoview of the rhodopsin structure from the three-dimensional crystals (PDB entry 1HZX). The polypeptide is represented as a yellow ribbon. N and C denote the N and C termini, respectively. Helical segments are labeled I–VIII. The retinal chromophore is shown in red. The figure was drawn by using the Molscript^[58] and Raster3d^[59] programs.

The transmembrane helices contain 56% of the amino acids in the protein. The remainder of the polypeptide is distributed among the N-terminal tail, the three extracellular loops between the helices, the three cytoplasmic loops, and the C-terminal tail. Several residues in two of the cytoplasmic loops and at the C terminus are mobile and not included in the molecular model.

11-*cis*-retinal serves as a cofactor for rhodopsin and the cone opsins. It is covalently linked to Lys296 in helix VII. The prosthetic group forms a Schiff base linkage with the amine group of the lysine residue. The retinal is located between the helices towards the extracellular side of the protein (see Figure 1) and is completely buried within the protein. The transmembrane helices block ready access to the hydrophobic region of the bilayer, and the β strands and extracellular loops shield the chromophore from the aqueous environment on the extracellular face. The binding site is some distance from the cytoplasmic surface and amino acid side chains from the transmembrane helices block access to that surface.

Figure 2 shows the superposition of bovine rhodopsin^[22] and the helical model available from low resolution cryo-EM of two-dimensional crystals of frog rhodopsin.^[10, 26] The transmembrane helices in the two structures have the same relative intramolecular locations, orientations, and sizes. However, there are no structural features in the rhodopsin model from the crystal structure analysis that permit an unambiguous determination of the molecular orientation within the membrane. Integral membrane proteins contain hydrophobic surfaces that interact with the hydrophobic interior of their membranes, but there is an uneven transition between that surface and the hydrophilic surface facing the aqueous environment outside the membrane. It has been noted before^[27] that the tryptophan residues in membrane proteins tend to be located at the crossover between hydrophobic and

hydrophilic surfaces. In the case of rhodopsin, that is not the case.^[28] Alternatively, the charged side chains in rhodopsin should be located outside the membrane, but defining the molecular orientation in the membrane on the basis of their location is difficult. It is easier, once the orientation of the molecule is known from the superposition of the crystallographic and electron-microscopic models, to define limiting planes just inside of the charged residues that probably mark the edges of the membrane. In this case, the planes would be 30 Å apart, a reasonable estimate of the thickness of the membrane, especially if its thickness adjusts to accommodate the transmembrane portion of the protein.

One interesting structural feature that became apparent with the 2.8 Å resolution structure was that the transmembrane helices are not straight, regular α helices. Both the cryo-EM and X-ray crystallographic structures show that the helices are kinked and bent, and the nature of the kinks has been analyzed elsewhere.^[28, 29] In addition, it has been noted that turns of 3_{10} and π helices are also found in this structure. In addition, in several of the helices, proline residues are associated with the kinks. Steric clashes between the proline side chain and the carbonyl group that would normally hydrogen bond to this position (in an α helix) force the carbonyl group to twist away from the helical axis. This alters the main-chain torsion angles in the neighboring residues, and often results in a bending or kinking in the helical axis. To relieve the close interatomic packings, the helix bends to provide room for the carbonyl group. While this is a reasonable explanation for several of the kinks, it is not the case for the kink in helix II at Gly89 and Gly90. There is no proline residue in this helix, but the helical axis bends by 30°.

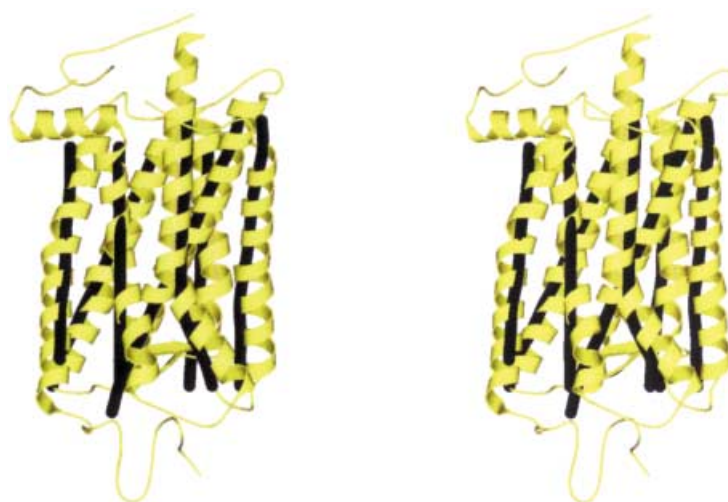


Figure 2. Stereoview of the rhodopsin structure from the three-dimensional crystals (yellow ribbons; PDB entry 1HZX) superposed on the helical axes of two-dimensional crystals from the cryo-electron microscopy study (black rods).^[26] The vertical axis is parallel to membrane normal. The figure was drawn by using the Molscript^[58] and Raster3d^[59] programs.

4. Retinal and Its Environment

Rhodopsin's chromophore, 11-*cis*-retinylidene, is located between the transmembrane helices, offset somewhat from the center of the membrane towards the extracellular side of the molecule. The 11-*cis* conformation is readily identified, even at 2.8 Å resolution.^[22] As known from spectroscopic studies of the protein, the chromophore is not planar.^[30] The conjugated system is twisted, possibly due to packing interactions with the protein, although steric interactions within the chromophore also contribute. Twisting of the chromophore might provide one way of adjusting the wavelength dependence of photon absorption.

The chromophore binding environment is made up of a mix of hydrophobic and polar/charged groups. Phe212 and Phe261 are located near the β -ionone ring, and the side chain of Trp265 is located midway between the ring and the Schiff base linkage to Lys296. The retinal is bent around this residue in the ground state. Polar groups such as Thr118 and Tyr268 are located near the center of the polyene, and the side chain of Glu122 is close to the β -ionone ring. Also, Glu183 makes a close water-mediated approach to the retinal. The Schiff base at the other end of the chromophore is protonated in the activated form of the protein, and Glu113 serves as a counterion for it in the interior of the protein. More detailed descriptions of the retinal environment have been presented previously,^[3, 4, 25] including comparisons with information about the binding site obtained by using noncrystallographic techniques.

Cone opsins are proteins related to rhodopsin and are present in cone cells. These proteins again use 11-*cis*-retinal as a chromophore, but their absorption maxima are located at wavelengths different from that of rhodopsin.^[1] These changes introduce wavelength sensitivity to the photoreceptors and form the basis for color vision. The changes in the absorption maxima come about because of differences in the retinal environments in the cone opsins. Comparisons of the amino acid sequences for the human blue, green, and red cone opsins with that of rhodopsin show 41, 38, and 37% amino acid identities, respectively. Homology-modeling efforts for the cone opsins can take advantage of these high sequence identities to indicate how the amino acid substitutions known to affect the retinal environment actually do so. Initial efforts along these lines are being published,^[28] but further work in this area can be anticipated.

5. Rhodopsin and Other Known Membrane Protein Structures

Until the rhodopsin crystal structure became available, the archetypical structure for seven-transmembrane-helix proteins was that of bacteriorhodopsin.^[31] Bacteriorhodopsin pumps ions across bacterial membranes. Photon absorption by its retinal chromophore drives the pumping process, but absorption of a photon converts the all-*trans* chromophore into the 13-*cis* isomer. The protein's transmembrane helices are topologically identical to those in rhodopsin, but the retinal chromophore is attached to a lysine residue in helix VII that is not sequentially or

structurally equivalent to Lys296 in rhodopsin. Comparison of the structure of bacteriorhodopsin with that of rhodopsin has been made.^[25] If the molecules are aligned on the basis of their structures, 79 C α atoms in the transmembrane helices can be superposed with a root mean square distance of 2.13 Å between equivalent C α atoms. Five of the transmembrane helices in each protein are aligned, but residues in the other two helices (IV and V) differ in position by as much as 10–15 Å. The helices in bacteriorhodopsin are more regular in structure than the kinked helices in rhodopsin. As stated above, the retinal chromophores in the two molecules are covalently attached to lysine residues that are separated by two helical turns. Nevertheless, in the ground state, the β -ionone rings at the ends of the chromophores are close to each other when the structures are aligned by using only the C α atoms.

6. Rhodopsin and GPCR Function

The rhodopsin crystal structure provides an interesting view of a significant photoreceptor molecule, but just as importantly, it provides the first three-dimensional structure of a GPCR. The importance of GPCRs in biological processes^[17, 32–35] and their roles as drug targets have promoted interest in the rhodopsin structure as a model for understanding ligand binding and signaling processes in this class of receptors.

One structural feature relevant to the use of the rhodopsin structure as a base for the modeling of other proteins is connected with the kinks and bends in the helices. These distortions from ideal helical structure cause pieces of the helices to rotate relative to an ideal helix. This places residues in environments with different neighboring residues than would have been predicted by using other starting structures for homology modeling. Comparison with bacteriorhodopsin shows how significant this can be. At first view, the two structures are similar with the seven helices arranged in the same tertiary structure. Looking at the structural details in the helices, it becomes apparent that the two structures would predict quite different locations for the same residues when used in homology-modeling exercises. At this point, indications are that the rhodopsin model serves as a better starting point for modeling of other GPCRs. This is not a surprise, but how representative the rhodopsin transmembrane helices are for other GPCRs needs further study.

The retinal binding site is another area where the rhodopsin structure might prove useful in modeling other GPCRs. The hydrophobic chromophore is similar to the ligands bound by many GPCRs, and evidence is available to support the idea that the retinal site is representative of the ligand binding sites in those proteins.^[17, 18]

One aspect of the binding site that was surprising when the structure was first analyzed is that it is completely buried within the protein. The retinal site is not accessible to the cytoplasmic or extracellular surfaces. The site is farther from the extracellular than the cytoplasmic surface, and the packing of the helices simply fills the internal space in that direction. Towards the extracellular surface are found the two short β strands and

extracellular surface loops that make up the “plug” blocking access in this direction.^[36]

The retinal site is not accessible to the hydrophobic environment within the membrane either. No gaps between the helices present a path for the retinal to exit the binding site. Given the need for ligands to move in and out of the binding site and for retinal to do likewise in the regeneration of the photopigment, alterations in the published structure must occur in the functional protein. X-ray diffraction patterns are measured over relatively long time periods (hours) and large samples of molecules, so the structural views are temporal and spatial averages. Thermal fluctuations in the structure might be sufficient to allow the passage of ligands into and out of the binding site, but longer-lived structures with large open paths to the site are also possible.

The general features of the retinal site (location, orientation, environment) might be applicable in studies of other GPCRs and their ligands. This probably does not hold for the details about the site. Various derivatives of retinal, including some with major substituents, have been used to probe the properties of the chromophore and its environment.^[30, 37, 38] Various spectroscopic techniques, including NMR studies of wild-type protein and fluorinated derivatives^[39–41] are being applied to rhodopsin to characterize the dynamics of the chromophore and G-protein binding sites. These and earlier studies point out the flexibility of the binding site in accommodating ligands. This, and the fact that retinal is bound covalently in its site, will complicate efforts to model the binding of ligands in other GPCRs.

However, the structural model of ground-state rhodopsin provides some hints about the dynamic activation and signaling process of the receptor. A brief description of them is presented here. More details about the signaling process can be found in recent publications.^[4, 8, 42, 43]

Ground-state rhodopsin is inactive, and several of its structural elements combine to restrain the structure. Interactions among the extracellular loops, including a disulfide bridge,^[44–47] limit the conformation flexibility of this part of the molecule under dark, nonsignaling conditions. In addition, the interactions between the chromophore and the protein, both hydrophobic and electrostatic, tighten the inactive receptor structure. Mutations of Lys296 or Glu113 eliminating these interactions result in constitutively active receptors.^[48]

Three other parts of the rhodopsin structure are also important for holding it in the inactive form. One of these is a tight set of interhelical contacts involving Asp83, Asn55 in helix I, and Asn302 in the NPXXY region. Also, residues in the helix III in the center of the bundle of helices interact with residues in each of the other helices (except helix I). Finally, Arg135 (in a conserved D/ERY sequence motif) makes ionic interactions within the receptor that are important for holding the structure in the inactive form. Mutations in this motif, for example, changing Glu134 into Gln134, generate constitutive, hyperactive receptors.^[13]

The first step in the activation of rhodopsin is the photoisomerization of 11-*cis*-retinylidene into all-*trans*-retinylidene after absorption of a photon. This isomerization is fast,^[49] and slower conformational adjustments in the protein as well as the

chromophore eventually give rise to the signaling form of the protein, metarhodopsin II.

Cross-linking studies^[50] indicate that a large movement of the β -ionone ring is involved in forming metarhodopsin II. In addition, the conformational switching of the chromophore might require reorganization of the helical structure of helix VII. This leads to disruption of the salt bridge between Glu113 and Lys296^[48] and movement of a proton.^[51]

The conformational change in the chromophore gives rise to movement of the transmembrane helices^[43, 52] with helix VII moving away from helix I and helix VI moving away from the other helices. Glu247 is no longer able to interact with Arg135, which is then able to reorient to the cytoplasmic surface where it can interact with transducin. Protonation of Glu135 also occurs during this conformational transition.^[4]

Site-directed cysteine mutants have provided a means for obtaining structural information for activated rhodopsin. These have been utilized in studying disulfide cross-linking patterns as well as for providing sites for the introduction of spin labels.^[43, 52–55]

The rates of formation of cross-links depend on the distance between the cysteine residues, as do the dipole–dipole interactions between nitroxide spin labels attached to the protein at specific cysteine residues. Comparison of these distances before and after light activation of the protein shows which parts of its structure are altered in formation of the signaling state, metarhodopsin II. Spin labels have been introduced at positions 306, 313, and 316 in the rhodopsin sequence, and the changes observed at position 313 in going from the ground state to the activated protein can best be explained by movements of helix VII.^[55] In addition, the cytoplasmic ends of helices II and VI bend away from the center of the molecule, with helix VI moving the largest distance (about 8 Å).

This opening up of the cytoplasmic face of rhodopsin generates a binding site for interactions with and activation of transducin.^[7] Motion of helix VII has been identified as important for the interactions with the G-protein.^[56, 57]

Many of the conformational changes giving rise to the transducin binding site are part of a general mechanism for GPCR activation. The details of the initial signaling event, that is, absorption of a photon for rhodopsin and binding of ligands by other GPCRs, will of course differ within the protein family, but sequence similarities indicate that disruption of the interactions involving the D/ERY motif and the relative motions of the helices are not unique to rhodopsin.

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