G-Protein-Coupled Receptors for Light: The Three-Dimensional Structure of Rhodopsin

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KEYWORDS:

membrane proteins · receptors · rhodopsin · signal transduction · vision

G-protein-coupled receptors (GPCRs) form the largest known family among integral membrane proteins. Up to 5% of all genes encoded in the genomes of higher eukaryotes, such as the nematode *Caenorhabditis elegans*, belong to the GPCR superfamily and more than 84 billion US $ are spent per year on drugs that modulate GPCR function. Their highly conserved topology is made up of seven transmembrane helices and allows these membrane proteins to convert a plethora of different extracellular signals like hormones, pheromones, odorants, or light into the activation of intracellular, heterotrimeric G-protein complexes. Consequently, the vast amount of different binding and signaling specificities of GPCRs was often compared with the huge repertoire of binding specificities that is generated by the modularly arranged variable regions of antibodies. However, despite their widespread occurrence in humans and other eukaryotes and the tremendous importance of GPCRs in the pharmaceutical industry, until recently almost no structural information was available which was sufficiently resolved to reveal the mechanistic details of ligand binding and signal transmission through the lipid bilayer.

This situation has now changed through the advent of the first structure of a GPCR, the dim-light receptor rhodopsin ("red" opsin) whose structure was determined at 2.8 Å resolution from three-dimensional (3D) crystals by Palczewski and co-workers.[1] That bovine rhodopsin would become the first GPCR with known 3D structure was expected, because most structural studies on GPCRs so far focused on this visual pigment. Its central role in mammalian vision and the ease of its preparation from natural sources (for example, ca. 0.5 mg can be purified from a single bovine retina) are advantageous aspects for the biochemist. Furthermore, eukaryotic rhodopsins are highly representative for GPCRs, because they belong to class A of the GPCR superfamily, which covers 90% of all known GPCRs including pharmacologically relevant examples like the adrenergic and dopaminergic receptors.

Unlike other GPCRs, rhodopsin contains its built-in "ligand" retinal in a covalently bound form as a protonated Schiff base with a lysine residue (K296) in helix VII. Upon illumination ($\lambda_{max} = 500$ nm), this chromophore photoisomerizes from an 11-*cis* to an *all-trans* configuration in less than 0.2 ps (Figure 1). After a series of distinct photointermediates has been passed through (Figure 1), an active receptor conformation (R*) is formed by deprotonation of the protonated Schiff base, which corresponds spectroscopically to the blue-shifted metarhodopsin-II (meta-II) intermediate ($\lambda_{max} = 380$ nm). This R* state resembles the agonist-activated form of GPCRs and catalyzes the guanine nucleotide exchange reaction in the heterotrimeric G-protein transducin. In the rod cells of vertebrates, GTP-bound transducin then lowers the cGMP level by activation of a cGMP phosphodiesterase, which in turn causes a closure of cGMP-gated cation channels and a hyperpolarization of the plasma membrane. Overall, the photon capture by a single rhodopsin molecule effects a remarkable signal amplification as ca. 10^8 cations are prevented

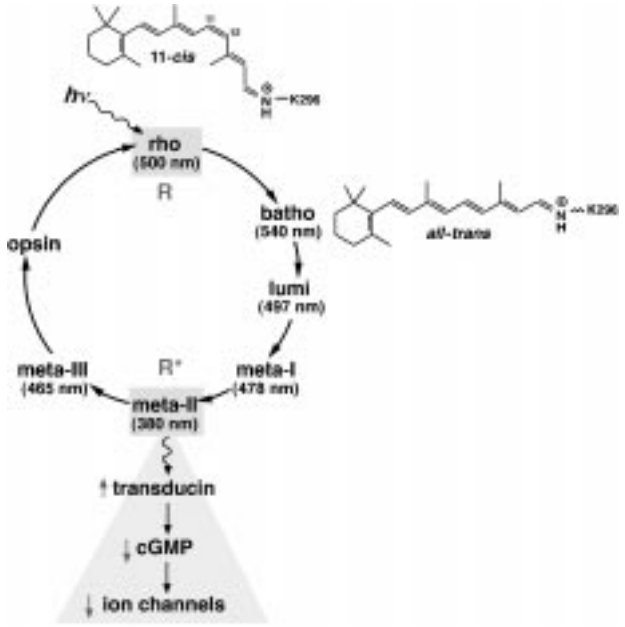

Figure 1. *The visual cycle in mammalian rod outer segments. The structure of rhodopsin (rho) determined by Palczewski et al. gives a first view on the initial state of rhodopsin prior to photoactivation (R). While this state should correspond to an antagonist-bound form of a GPCR, the metarhodopsin-II (meta-II) photointermediate resembles an agonist-activated species (R*).*

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from entering the cell through the plasma membrane. Analogous to other GPCRs, metarhodopsin-II becomes a target for a parallel pathway that quenches the active R* state and consists of phosphorylation by a rhodopsin-specific protein kinase, subsequent binding of visual arrestin, and dephosphorylation.

Due to the multitude of conformational states which rhodopsin adopts for its function, it was not clear whether well-ordered 3D crystals of a mammalian rhodopsin could ever be obtained by using standard procedures. A primary experimental hurdle is certainly the requirement for detergents during purification and crystallization, which compromises the stability of the solubilized protein species. Additionally, rhodopsin is subjected to numerous posttranslational modifications including glycosylation at the asparagine residues N2 and N15, palmitoylation at cysteines C322 and C323, disulfide formation between cysteines C110 and C187, acetylation at methionine M1, and the light-dependent phosphorylation at serine and threonine residues of the C-terminal tail. Last but not least, rhodopsins from vertebrates are extremely light-sensitive, because these rhodopsins are destined to be hydrolyzed to the apoprotein opsin and free *all-trans*-retinal after formation of metarhodopsin-II (Figure 1). In vivo, this bleaching process makes sense, because 11-*cis*-retinal is regenerated in the adjacent retinal pigment epithelium. However, in vitro all steps including the purification from retinae, crystallization, and crystal handling had to be performed under dim red light to keep the holo form of rhodopsin intact.

Due to the obstacles to obtain 3D crystals of eukaryotic rhodopsins,[2] until recently structural data were only derived from two-dimensional (2D) crystals whose formation from intact membranes of rod outer segments was demonstrated by Corless and co-workers in the early eighties and optimized by Schertler and his colleagues during the nineties.[3] Electron crystallographic studies by the latter group initially proved the postulated architecture of a seven-transmembrane-helix (7TM-helix) bundle for the vertebrate rhodopsins. Using electron crystallographic data from the 2D crystals of frog rhodopsin which extended to 7.5 Å in the membrane plane (but only to 17 Å resolution perpendicular to the membrane) it became even possible to derive a reliable packing model of the 7TM-helix bundle that was later extensively used in homology modeling studies of the transmembrane domains of other GPCRs. Interestingly, the breakthrough in the 3D crystallization of bovine rhodopsin now reported by Palczewski et al.[1, 4] did not benefit from advances in the crystallization methodology of membrane proteins itself, which were crucial for the successful structure determinations of archaeal rhodopsins during the previous decade.[5] Rather, it was the discovery of a new protocol for the selective extraction of bovine rhodopsin from rod outer segments by Okada et al. that led to preparations yielding 3D crystals useful for X-ray crystallographic analysis.[4, 6] One unusual component of the extracting agents, zinc ions used in high concentrations, was observed at two positions in the refined X-ray crystallographic structure of rhodopsin where it stabilized the overall protein fold. This finding again suggests that additional stabilization of membrane proteins, as already applied in the cocrystallization of respiratory chain enzymes with antibody fragments,[7] might be essential for further progress in their crystallization.

As explained below, the work of Palczewski et al.[1] clearly confirms that the eukaryotic family of rhodopsins evolved independently of the well-characterized archaeal family which includes light-driven proton and halide ion pumps like bacteriorhodopsin or halorhodopsin.[5] Only topological features are common to both families, such as the presence of seven transmembrane helices, a retinal chromophore that is linked as a protonated Schiff base to a lysine residue in helix VII, and an acidic residue, E113 in bovine rhodopsin, that acts as a counter charge and proton acceptor on helix III. Apparently, there are only a few choices in nature for membrane-bound receptors to select and accommodate a photoisomerizable and lipid-soluble ligand, which might explain why retinal was incorporated as the chromophore in two structurally distinct protein families.

In bovine rhodopsin, the regions which protrude from the lipid bilayer are much larger and more organized than in archaeal rhodopsins (Figure 2) as they carry the functionally most important sites, the G-protein-binding site on the cytosolic side and parts of the retinal-binding pocket on the extracellular side. Furthermore, the retinal-binding pocket does not reside in the center of the transmembrane region, but is unexpectedly located near the extracellular side of the 7TM-helix

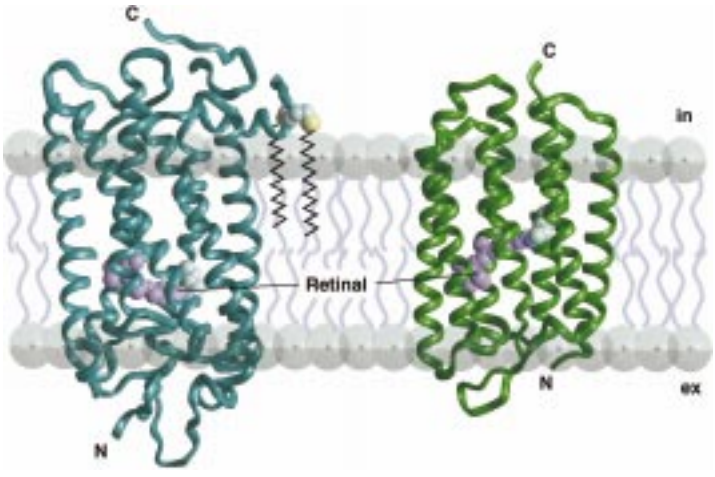

Figure 2. *Two 7TM-helix proteins in their membrane context. The extra mass of mammalian rhodopsins (left, bovine rhodopsin: 348 amino acids) as compared to archaeal rhodopsins (right, bacteriorhodopsin: 248 amino acids) is mainly located in the extramembranous loop regions (in = intracellular side, ex = extracellular side). Palczewski et al. report electron density for the two palmitates which are thioesterified to cysteines C322 and C323 (the approximate positions of the fatty acid chains are indicated by line drawings).[1] The resulting fourth cytosolic loop folds onto an amphiphilic α helix that runs almost parallel to the membrane plane and is postulated to participate in G-protein binding.*

bundle. For this reason, the N terminus and the loop between helices III and IV fold into a four-stranded β sheet that invaginates the 7TM-helix bundle to wall parts of the retinal-binding site. Due to the higher inclination angles of the transmembrane helices with respect to to the membrane plane, the arrangement of the helices is distinct from the straight helix bundles observed in archaeal rhodopsins. Surprisingly, with the exception of the central helix III, all transmembrane helices are kinked, mostly due to the presence of prolines or, as in helix II, a Gly-Gly motif. In the archaeal rhodopsins, such distortions of the regular α-helical pattern in the transmembrane parts occur mostly next to the sites of the largest structural changes, for example, at the Schiff base lysine. It will be interesting to see whether in mammalian rhodopsins and other GPCRs, a hinge-like motion in the 7TM-helix bundles is required for a long-range communication between the ligand and G-protein-binding sites.

The large spatial separation of ca. 40 Å between the Schiff base nitrogen atom and the cytosolic surface of the G-protein-binding site (Figure 3) might also be a reason why eukaryotic rhodopsins use an 11-*cis* →*all-trans* photoisomerization for their retinal chromophore instead of the *all-trans* →13-*cis* isomerization found in archaeal rhodopsins. The central location of the C11–C12 bond would result in a large swivel motion of the ionone ring upon photoisomerization which might be transmitted into large-scale structural rearrangements of bovine rhodopsin. In contrast, the photoisomerization of the C13–C14 bond in the retinal of archaeal rhodopsins has no major effects on the location of the ionone ring itself, but triggers only small, local structural changes, which ensure the unidirectionality of ion transport.

In humans and many other animals, rhodopsin is only responsible for dim-light perception. The color vision of humans is mediated by a set of homologous red, green, and blue visual pigments ($\lambda_{max} \approx 560$ nm, 530 nm, 425 nm, respectively), which are located in the cone cells of the retina. The structure of the retinal-binding site now presents an amazing insight into how these different pigments perform spectral tuning over a wide wavelength range. For example, the main differences between the red and green pigments from cone cells originate from a small number of amino acid exchanges which occur in close proximity to the polyene system of the chromophore (Figure 3). The introduction of hydroxy groups at these positions by Phe →Tyr or Ala →Ser/Thr exchanges, respectively, causes a green-to-red opsin shift, most likely due to dipole – dipole interactions of the hydroxy groups with the excited state of the chromophore. The structure reported by Palczewski et al.[1] suggests another component of spectral tuning. This is the perturbation of the retinal chromophore by a twisted conformation of the retinylidene group. At the moment, the resolution of the rhodopsin 3D crystals is still not sufficient for an unambiguous assessment of the chromophore conformation without using a priori chemical knowledge during the crystallographic refinement. More precise structural information about the retinal conformation should be expected in the near future from solid-state NMR spectroscopic studies of rhodopsin, although some recent reports gave contradictory structural interpretations, which might reflect differences in the NMR methodology used or in sample preparation.[8] Nevertheless, this knowledge will be of considerable interest for quantum chemists who want to correlate the energetics between the protein environment and its bound retinal chromophore with the observed spectral tuning.

Unfortunately, the most characteristic region of GPCRs, the G-protein-binding site, is still ill-defined in the X-ray crystal structure of rhodopsin due to excessively high temperature factors and the incomplete tracing of the polypeptide chain in the loop between helices V and VI and the C-terminal stretch.[1] Even if further crystallographic refinement of this crystal form will improve the identification of the G-protein-binding site, its conformation might be of limited value for understanding, in structural terms, how GPCRs catalyze the guanine nucleotide exchange in G-protein complexes, because various studies demonstrated significant conformational changes of the binding site upon G-protein binding and GPCR activation. For example, structural changes upon photoactivation of rhodopsin expose an epitope at the C-terminal end of helix VII that becomes accessible to a monoclonal antibody only in the meta-rhodopsin-II intermediate.[9] Other studies using ^{19}F NMR spectroscopy or site-directed spin labeling likewise gave evidence for directed movements of the adjacent ends of helices III and VI.[10]

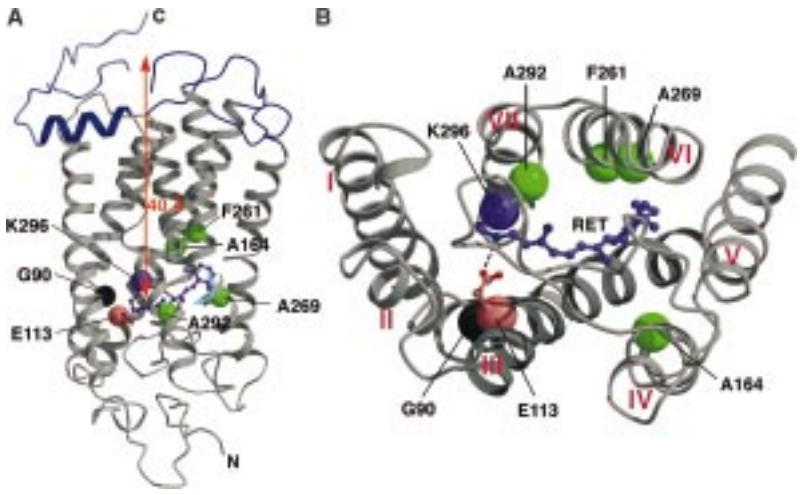

Figure 3. *The retinal-binding site of bovine rhodopsin (A: side view; B: top view). The side view shows the large spatial separation between the G-protein- (blue) and retinal-binding sites. Swivel motions of the ionone moiety of retinal, which might occur upon photoisomerization, are indicated by an arrow (cyan). Residues that are known to be important for the color tuning of red and green pigments in cone cells contact the retinal chromophore directly (green spheres: F261, A269, A292) or mediate their tuning effect indirectly (A164) through an intervening glutamic acid residue (E122). The disruption of the salt bridge between K296 and E113 (dashed line) is known to accompany activation of rhodopsin. Mutations of these residues or of G90 nearby are found in patients with autosomal dominant retinitis pigmentosa or congenital stationary night blindness.*

Finally, what might be the other major spin-offs of this first GPCR structure apart from drafting a molecular mechanism of vision? To the computational biologist who is interested in modeling pharmacological targets of the GPCR superfamily, the bovine rhodopsin structure provides a first reliable framework for rational drug design. Nevertheless, severe difficulties will still be encountered in modeling the small-molecule-binding sites in the 7TM-helix bundles of other class A GPCRs, because the retinal-binding site of rhodopsin is obviously also formed by irregular loop regions which are difficult to predict. Furthermore, until a structure of the metarhodopsin-II state of rhodopsin is determined, we will not know exactly how structural rearrangements in the ligand-binding site are accompanied by changes in the G-protein-binding site. However, the latter is a prerequisite for the structure-based design of compounds that act either as agonists or antagonists in GPCR-mediated signaling. For the biochemist and biophysicist, the rhodopsin structure will enable more sophisticated studies on monitoring the transition between active and inactive states of GPCRs. Last but not least, for the structural biologist, the structure of Palczweski and co-workers points the way toward a structure determination of the complex between a GPCR and its cognate G-protein.

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