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Opsin, a Structural Model for Olfactory Receptors?**

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The large family of G-protein-coupled receptors (GPCRs) detects signaling molecules such as hormones, neurotransmitters, and odorants. About half of the GPCRs are olfactory receptors (ORs), for which high-resolution structures remain elusive. ORs belong to the family of rhodopsin-like GPCRs. Rhodopsins are the photoreceptors in vision, and their ligand, retinal, shares high hydrophobicity with OR ligands. Herein we report a new crystal structure of the opsin apoprotein, that is, retinal-free rhodopsin, from the disc membrane of vertebrate retinal rod cells. A molecule of octylglucoside was identified in the ligand-binding pocket, where it replaced retinal and stabilized the active receptor conformation. The surprisingly well-defined hydrogen-bond pattern, which holds octylglucoside in the ligand-binding pocket was reminiscent of the dynamic hydrogen-bond pattern proposed for OR–ligand interaction, in which the receptor offers changing side chains for bonding.[1] Further, experiments with various detergents resulted in a defined capability to occupy the ligand-binding pocket of opsin. These findings indicated that the interaction of detergents with opsin may provide a simple model that mimics OR activation. The study provides a possible way for hydrophobic odorants to enter ORs, supports hydrogen bonding between odorant and receptor for OR activation, and recommends opsin as a suitable basis for OR homology modeling.

GPCRs, also referred to as seven-transmembrane helix (7TM) receptors, comprise seven α-helical segments that span the cell membrane to sense and relay extracellular signals into the cell. Binding of signaling molecules such as hormones, neurotransmitters, odorants, or drugs changes the equilibrium of GPCR conformational states and thereby initiates coupling to intracellular G-protein- and arrestin-mediated signaling pathways.[2] Understanding the mechanism of GPCR activation as well as structure-based drug design require high-resolution GPCR models.[3] Ligand-bound structures are emerging for rhodopsin-like GPCRs, the largest GPCR family with about 700 members in humans.[2a,4] ORs, crucial for the sense of smell, account for more than half of this family, but difficulties in overexpression limit OR characterization and make high-resolution structure analysis impossible to date.[5] Therefore, homology modeling based on rhodopsin is typically used for predicting ligand–receptor interactions.[1,6]

For GPCRs, it was found that the quality of prediction relies upon closely related homology modeling templates, and the possibility to include biochemical and quantitative structure–activity relationship data.[7] The known nonrhodopsin GPCR structures feature ligand-binding pockets that open towards the extracellular space to harbor water-soluble ligands.[2a] In the sphingosin 1-phosphate receptor with a bound sphingolipid mimic, the binding site is largely

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occluded by a cytoplasmic loop.[8] In rhodopsin, the hydrophobic ligand retinal binds deep inside the protein and has no access to the aqueous phase (Figure S1 in the Supporting Information).[9] According to binding-site predictions, hydrophobic odorants appear to bind similarly deep inside ORs,[1,6a–c] which suggests that rhodopsin is a better structural template for homology modeling than available crystal structures of nonrhodopsin GPCRs. The structure of inactive rhodopsin, however, may not be ideal, because it does not show an entrance for retinal. A potentially useful template for olfactory agonists may be the structure of the rhodopsin apoprotein, opsin, in its active conformation, Ops*.[10] Our work shows that opsin has the potential to serve in studies aimed at the molecular mechanisms of ORs.[1,6d]

The ability of GPCRs to adopt multiple conformations is key to their biological function; extracellular ligands stabilize specific GPCR conformations to which G proteins and arrestins couple and initiate intracellular signaling.[11] For opsin it was surprising that the crystal structure of the retinal-free GPCR represents the active Ops* state, because opsin in its native membrane is orders of magnitude less active towards its G protein than the metarhodopsin II (Meta II) state from which it arises by retinal release.[12] An explanation was provided by proposing a pH-dependent equilibrium of inactive and active opsin states in native membranes.[13] Further to lowering the pH value, replacing the membrane by certain detergents favors the formation of active conformations of rhodopsin upon photoactivation.[14] Solubilization of opsin in the detergent *n*-octyl β-D-glucopyranoside (OG) and crystallization at pH 5.6 was therefore considered to promote the formation of Ops* in the crystal. The Ops* conformation found under these conditions represents an active G-protein-interacting state as confirmed by the crystal structure of Ops* in complex with a synthetic peptide (GαCT), which is derived from the key GPCR binding site on its heterotrimeric G protein, the C-terminal region of the α subunit.[15] This view was further corroborated by the structure of Meta II, which could be obtained by soaking Ops* crystals with its agonist, all-*trans*-retinal,[16] or by crystallizing a light-activated constitutively active rhodopsin mutant.[17] In addition, the overall structures of Ops*–GαCT and agonist-bound β_2-adrenergic receptor in complex with Gs, the stimulatory G protein that activates adenylyl cyclase, are highly similar.[18]

The resolution of prior Ops* structures (ca. 3 Å) did not allow interpretation of patches of residual electron density in the retinal-binding pocket (Figure S2 in the Supporting Information). We have now determined the crystal structure of Ops* bound to a slightly modified G protein peptide (GαCT2) at a resolution of 2.65 Å and found clear electron density representing one OG molecule residing in the retinal-binding pocket of Ops* (Figure 1, Figures S3 and S4 and Table S1 in the Supporting Information). The overlay of the present Ops*–GαCT2–OG structure with Meta II–GαCT2 shows a nearly identical polypeptide chain with a Cα root mean square deviation (r.m.s.d.) value of 0.28 Å and only small changes in the conformation of side chains in the retinal-binding pocket (Figure 1a, Figure S5 in the Supporting Information). Moreover, OG occupies almost the same

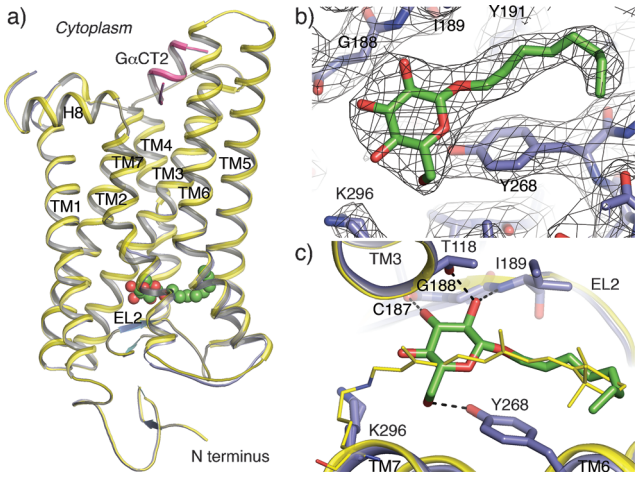

Figure 1. Comparison of Meta II–GαCT2 and Ops*–GαCT2–OG. a) Overlay of Meta II–GαCT2 (yellow, protein data bank (PDB) ID: 3PQR) and Ops*–GαCT2–OG (gray) structures shown in cartoon representation with peptide GαCT2 (magenta). The OG molecule in the retinal-binding pocket is shown as a red and green sphere model. b) OG molecule (green and red) in the retinal-binding pocket as stick model with $2F_o-F_c$ electron density map contoured at 1.0 σ (black mesh). c) Overlay of Meta II–GαCT2 (yellow) and Ops*–GαCT2–OG (blue) structures. For Ops*–GαCT2–OG side chains are shown as stick models, with two rotamer states for Lys296. The OG molecule is shown in red and green. For comparison, retinal bound to Lys296 in Meta II–GαCT2 is shown as thin yellow sticks. Dotted lines represent potential hydrogen bonding with bond length of 3 Å or less. Cys187-Gly188-Ile189 are located on extracellular loop 2 (EL2). Some residues are omitted for clarity. For water bound in hydrogen-bonding networks see Figure S5 in the Supporting information.

space as retinal, but with its ring moiety pointing into the opposite direction when compared to the cyclohexene ring of retinal; it lies next to Lys296 where the glucose ring is kept in place by hydrogen bonding to Thr118 on TM3, Tyr268 on TM6, and Cys187 and Ile189 on extracellular loop 2, which connects TM4 and TM5 (Figure 1b,c). The uninterpretable electron density in published Ops* structures, which we now identify as OG, might be due either to low occupancy of OG in the retinal-binding pocket or resolutions too low to trace the full OG molecule. A significant affinity of OG for the retinal-binding pocket would explain why Ops* crystals had to be soaked in a saturated all-*trans*-retinal solution for several hours to reconstitute Meta II.[16] This agrees with the finding that reconstitution of OG-solubilized opsin with 11-*cis*-retinal to form rhodopsin is very slow even in the presence of a large excess of retinal,[16] whereas reconstitution in *n*-dodecyl β-maltoside (DDM) occurs readily.[19]

The Ops*–GαCT2–OG structure showed a channel through the protein connecting two openings towards the surrounding lipid bilayer, one between TM5 and TM6, the other between TM1 and TM7 (Figure 2). Based on the Ops* structure and docking experiments, such a channel was described earlier for retinal, revealing several possibilities to dock this ligand, unless retinal is fixed as in rhodopsin by its Schiff base linkage to Lys296 on TM7.[10,20] Dynamic binding modes were also suggested for odorants in ORs.[1] We therefore became interested in investigating whether other

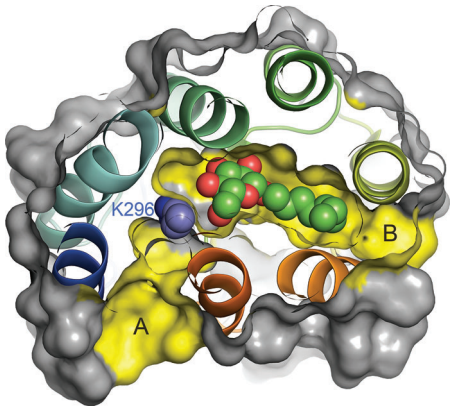

Figure 2. Ligand channel in Ops*–GαCT2–OG. In Ops* the retinal-binding site has two openings (A and B) towards the surrounding lipid bilayer for uptake of retinal[20,22] and other ligands like OG, respectively. Modeling studies have suggested that the two openings are connected by a channel (highlighted in yellow), which is constricted by the flexible side chain of Lys296. Lys296 and OG molecule are represented as gray/blue and green/red spheres, respectively. TM1 to TM7 are shown in rainbow colors (blue to orange).

parallels in ligand recognition exist between opsin and ORs. Recognition of odorants by ORs can depend on specific features of the odorant as for example, the length of the aliphatic hydrocarbon chain and the presence of heterocycles or specific functional groups.[21] We therefore measured reconstitution of rhodopsin spectroscopically at 500 nm in the presence of different detergents. For comparison with odorants, the sugar headgroup and the hydrocarbon chain length were varied (Figure 3, Figures S6 and S7 in the Supporting Information).

The detergents comprised *n*-alkyl β-D-glucopyranosides, *n*-alkyl β-maltosides as well as lauryl maltose neopentyl glycol and octyl glucose neopentyl glycol. Before reconstitution was started by addition of 11-*cis*-retinal, DDM-solubilized opsin was diluted to 3 mM DDM and incubated with different concentrations of the detergent to be tested (up to a 1250-fold excess over retinal). Decylmaltoside (DM) showed strong concentration-dependent inhibition of reconstitution, whereas this effect became smaller when the alkyl chain length was increased or decreased, with almost no inhibitory effect for tridecylmaltoside (TDM), DDM, and hexylmaltoside (HxM, Figure 3a,b). Also, the two neopentyl glycol detergents, which contain two sugar headgroups and two hydrocarbon chains, had little effect on rhodopsin reconstitution. This suggests that the tested alkylmaltosides, but not the much bulkier neopentyl glycol detergents, can enter the retinal-binding pocket. DM has a similar size as retinal and seems to fit best into the retinal-binding pocket. When the same experiment was performed with nonylglucoside (NG), OG, and heptylglucoside (HpG), inhibition was observed to depend on the hydrocarbon chain length and concentration with maximal inhibition above the critical micellar concentration of the particular detergent (Figure 3c, HpG: ca. 70 mM, OG: ca. 20 mM and NG: ca. 6.5 mM). Inhibition by OG was observed at somewhat higher concentrations when opsin was directly solubilized in OG instead of DDM as in the other experiments. When OG-solubilized opsin was diluted below the critical micellar concentration with DDM (3 mM), full reconstitution capability was recovered, thus showing that inhibition of reconstitution was the result of reversible OG binding in the retinal-binding pocket and not of a possible artifact such as protein aggregation. Interestingly, 200 mM maltose or glucose did not show any inhibitory effects.

We conclude that the tested alkylmaltosides and alkylglucosides can enter the retinal-binding pocket, especially when the concentration is above the critical micellar concentration. The affinity of the detergent for the retinal-binding pocket depends on steric constraints imposed by the alkyl chain, which affects the location of the sugar moiety in the retinal-binding pocket and thus the ability to form hydrogen bonds. Glucose and maltose had no effect on rhodopsin reconstitution, thus making it likely that the hydrophobic moiety of the detergent is necessary for entering the protein or at least contributes significantly to its affinity. The small inhibitory effect seen by the neopentyl glycol detergents may be attributed to a blocking effect on the openings to the retinal-binding pocket (Figure S8 in the Supporting Information).

From the tested detergents, OG, NG, and DM appear to assume a geometry that best fits the retinal-binding pocket of

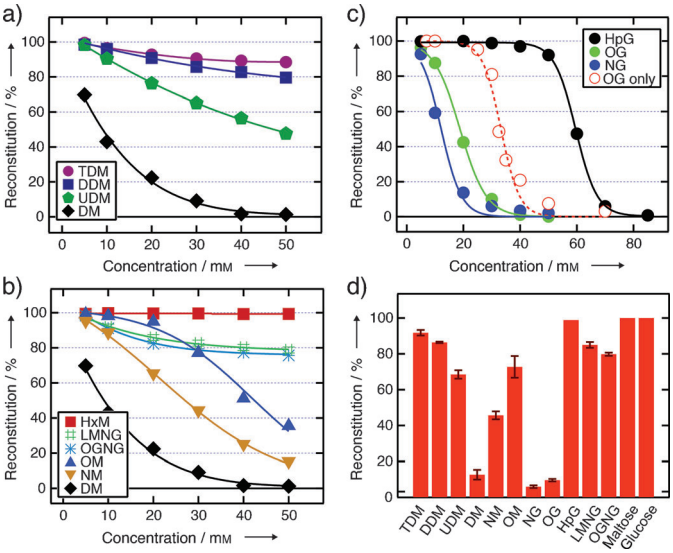

Figure 3. Reconstitution of rhodopsin from opsin and 11-*cis*-retinal in the presence of various detergents. Strong inhibition of reconstitution indicates the presence of detergent in the retinal-binding pocket. a, b) *n*-Alkyl β-maltosides (tridecyl-, TDM; dodecyl-, DDM; undecyl-, UDM; decyl-, DM; nonyl-, NM; octyl-, OM; hexyl-, HxM) or lauryl maltose neopentyl glycol (LMNG), octyl glucose neopentyl glycol (OGNG). c) *n*-Alkyl β-D-glucopyranosides (octyl-, OG; nonyl-, NG; heptyl-, HpG). Opsin was solubilized with 30 mM DDM or 50 mM OG (graph labeled "OG only"), diluted 10 times to 4 μM opsin with detergent at concentrations indicated in the plots. Rhodopsin formation after addition of 11-*cis*-retinal was measured at the absorption maximum of rhodopsin at 500 nm. d) Rhodopsin reconstitution in the presence of 30 mM detergent or 200 mM glucose or 200 mM maltose. Error bars represent standard deviations estimated from three independent experiments. The effect on reconstitution is dependent on both the nature of the headgroup and the alkyl chain length. The combinations found in DM, NG, and OG have the strongest effect.

Ops* and allows anchorage of the sugar moiety by multiple hydrogen bonds. Although it is known that OG can bind in peripheral pockets of membrane proteins,[23] it is surprising that an amphiphilic detergent molecule can replace the hydrophobic retinal, bind in the orthosteric ligand-binding pocket of opsin, and control the GPCR conformation by selecting the Ops* conformer from the conformational equilibrium.

The salient result of this study, however, is that opsin shows a number of features that are expected for ORs. Opsin can host not only various artificial hydrophobic retinal analogues as well as naturally occurring hydroxy analogues,[24] but also chemically distinct molecules like detergents containing multiple hydroxy groups. Ligand recognition in olfaction is thought to depend on the formation of a defined dynamic receptor–ligand hydrogen-bond pattern, with odorants having usually one functional group capable of serving as hydrogen bond donor or acceptor.[1] In the Ops*–GαCT2–OG structure hydrogen bonding to the ligand is observed with the multiple hydroxy groups of the tested OG ligand, engaging all hydrogen-bonding options at once. Although OG is more hydrophilic than odorants, its sugar moiety occupies a space in the ligand-binding pocket that is normally occupied by a hydrophobic part of retinal. The present Ops* structure also illustrates how hydrophobic odorants after partitioning into the membrane can enter from the lipid bilayer into the 7TM scaffold. Taken together Ops* may serve as the best currently available structural template for homology modeling of ORs and improved binding-site prediction for hydrophobic odorants. Improved OR homology models can help to decipher the molecular mechanisms that underlie our sense of smell, and future theoretical and experimental studies on opsin can help to address some of the challenging issues in olfaction, including the current controversy whether olfaction relies solely on recognition of ligand shape or has in addition a molecular vibration-sensing component.[6d,25]

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