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Molecular modelling of the complex of oligomeric rhodopsin and its G protein

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Abstract

The TM4–TM5 model of higher-order structure of rhodopsin (a structural and functional template for G protein-coupled receptors, GPCRs) in the membrane was used to investigate a complex between oligomeric rhodopsin (Rh) and its G protein (transducin, Gt). Comparison with currently available models of oligomeric organization of GPCRs was made, and is discussed with experimental data which revealed a flexible intradimeric Rh interface. We also characterized the membranous anchor domain of Gt in the complex Rh–Gt, which is critically important for the binding of transducin to Rh.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

G protein-coupled receptors (GPCRs) form a superfamily of receptors essential for signalling across plasma membranes [1–3]. In humans about 1000 genes encode GPCRs, with half of them being odour and taste receptors and the rest being receptors of endogenous ligands and light [4, 5]. Each GPCR responds to an extracellular stimulus by activating a specific G protein. Then, the trimeric G$\alpha\beta\gamma$ protein dissociates into Gα and G$\beta\gamma$ and one of them (depending on the GPCR) modulates specific enzymes that produce second messenger small molecules giving rise to a highly amplified signalling cascade. Such processes are responsible for vision, taste and smell, and also involve responses to many specific ligands like peptides, hormones, proteases, chemokines and others. Because of such a broad range of activities the GPCRs are important pharmacological targets [6], and a large part of drug research is focusing on them [7].

GPCRs are ubiquitous in cell membranes and critical in modulating virtually all physiological processes. These receptors share a similar structural architecture consisting of a bundle of seven transmembrane α-helices. The active conformations of the receptors are

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stabilized by binding of agonists. After rearrangement of receptor structure a cytoplasmic active site is formed ready to activate heterotrimeric G proteins. The family of G proteins is also structurally highly conserved. Cloning experiments estimated the human G proteins to consist of 35 genes, 16 encoding α-subunits, five beta and 14 gamma [8]. They function as GTPases exchanging guanine nucleotide and working as on–off switches. Because of the smaller number of G proteins than GPCRs, different GPCRs can activate the same G protein type, but also, one GPCR can activate several G protein types. This suggests that not only the receptor structure but also the process of signal transduction is very similar among GPCRs and could be extensively studied even on a single receptor subtype.

Phototransduction represents an excellent model system for understanding G protein signalling. Rhodopsin (Rh) is still the only GPCR with known three-dimensional structure since it was crystallized in 2000 [9], so it is the template for homology modelling of other GPCRs. An activation of Rh is done by absorption of a single quantum of light, which changes the conformation of retinal covalently bound to opsin (rhodopsin without its nonprotein part). Highly strained retinal slowly changes the structure of Rh (millisecond timescale compared to 200 fs for photoisomerization of retinal) starting the signalling process.

Rh proved to be useful also in studying dimerization of GPCRs. Currently it is believed that most GPCRs exist and act as dimers. It is even suggested that most GPCRs spend their whole life cycle in a cell as dimers (both homodimers and heterodimers), starting from formation of dimers in the endoplasmic reticulum with the help of dimer-probing cytosolic chaperons and ending with the internalization of these receptors. The recent large number of papers devoted to dimerization of GPCRs is summarized in several reviews [10–12]. The potential role of GPCR dimers in biosynthesis and maturation is reviewed in [13]. The recognition that GPCRs may form dimeric or oligomeric structures is based on a number of different biochemical and biophysical approaches. Although much effort has been spent to elucidate the mechanisms by which GPCRs interact with each other, the functional role of GPCR oligomers remains to be established. Additionally, the formation of heterooligomers of GPCRs greatly increases the possibilities of joint signalling that is to be revealed. The functional and pharmacological effects of the oligomerization of GPCRs are analysed in several reviews [14, 15]. The role of oligomerization in signal transduction is reviewed in [16, 17] and in drug design in [18]. However, a caution is needed because it was published recently that although the neurokinin-1 receptor tends to concentrate in microdomains it does not form constitutive or ligand-induced homodimers or oligomers [19]. Oligomerization of Rh is also questioned [20].

In this work, we analysed the stability of the G protein–Rh complex that considers full molecular dynamics (MD) simulations using a periodic box, in the membrane typical for flat discs from rod outer segments where Rh is located. To our knowledge only two full structure models showing structural organization of the complex of oligomeric Rh and trimeric G protein have been published. So far, they were not characterized by full MD simulations in periodic conditions. They employ different organization of Rh in the oligomeric assembly. The first one [21] is based on the TM4–TM5 interface of Rh dimer. The interface in the second model [22] is formed by helices TM1–TM2–TM3. Both oligomeric representations of Rh fulfil structural requirements from atomic force microscopy (AFM) measurements [23, 24] but only the first one was confirmed by recent crosslinking experiments [25, 26]. Based on this model of Rh oligomer (protein data bank accession code 1N3M) we continue to reveal structural details about this critically important interface between Rh and its G protein (transducin, Gt). Apart from stability issues of the whole complex we analysed the structure and interactions with lipids of post-translational hydrophobic modifications of proteins in the complex. They are important since the membranous anchor of Gt was found to be absolutely necessary for the binding of transducin to activated Rh [27].

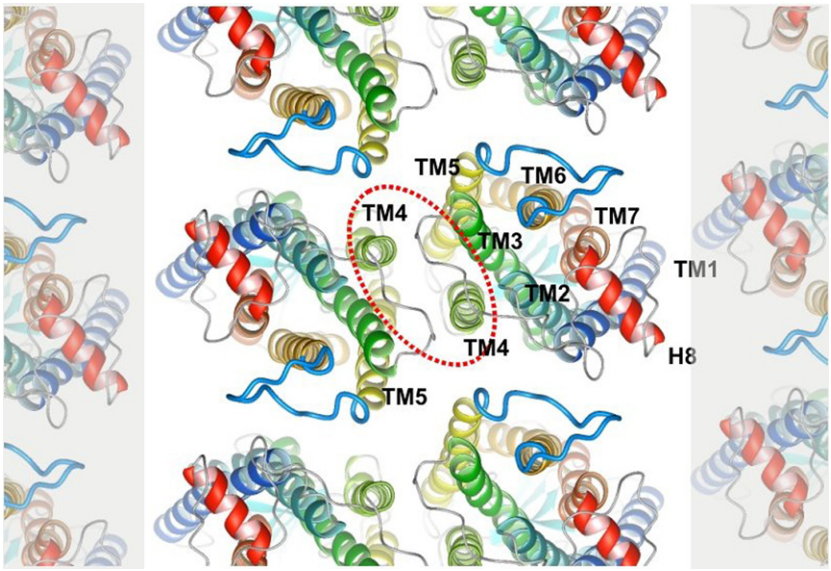

Figure 1. The model of the structure of rhodopsin oligomer based on the TM4–TM5 dimeric interface (PDB 1N3M). View from cytoplasmic side. The dashed ellipse marks the intradimeric interface. The long cytoplasmic loop between TM5 and TM6 is made bold. Adjacent double rows are greyed. The loop between TM5 and TM6 forms an interface between rhodopsin dimers. Contacts between double rows are formed by extracellular parts of TM1.

2. Models of Rh oligomers

Based on images of Rh oligomer in native membranes of rod outer segments done by AFM [23] it was possible to estimate geometric constraints in order to build a full atom model of the oligomeric organization of rhodopsin. AFM revealed that rhodopsin is densely packed as double rows in the form of a paracrystal. The most probable explanation was that Rh pairs forming double rows are dimers. The distance between rhodopsin monomers in a dimer was estimated to be 3.8 nm, which is also the distance to the closest rhodopsins in adjacent dimers. Double rows of rhodopsins were located every 8.4 nm. Using geometric constraints together with molecular modelling, the model of Rh oligomer was built. In this model the Rh–Rh interface is formed by two transmembrane helices TM4–TM5. This interface is depicted in the broader context of Rh oligomer in figure 1. The model of such an oligomer was deposited in the Protein Data Bank (PDB) as 1N3M [23]. The single unit in such an oligomer is a dimer forming long double chains. The choice of Rh–Rh interface determines the rest of the contacts in the oligomer: the dimers contact each other by a long cytoplasmic loop between helices TM5 and TM6; monomers of Rh from adjacent double rows are also in contact using the extracellular part of TM1. Such a model of an oligomer was checked to be energetically stable [24] and was enhanced later by addition of the membrane [21].

The second model of Rh oligomer based on the same AFM constraints was built by Ciarkowski *et al* [22] to study the binding of transducin to rhodopsin. It was based on a completely different Rh–Rh interface involving TM1, TM2 and TM3. It was derived from the 1N3M model by rotating Rh monomers clockwise by 90°. Contacts between adjacent dimers are formed by TM1 and cytoplasmic helix H8 from one dimer and TM4 with TM5 from the second dimer. Adjacent double rows contact each other by the cytoplasmic loop between TM5 and TM6.

The third model of Rh oligomer was based on low-resolution cryo-electron microscopy of two-dimensional crystals of squid Rh [28]. The Rh–Rh interface is formed only by TM4 from both monomers. This interface was confirmed by crosslinking data on D_2 dopamine receptors [29]. It was also found that the interface is dependent on ligand binding. Although the TM4–TM4 interface involves the same transmembrane helix as in the TM4–TM5 interface it uses another face of TM4, which is close to TM3, and as a result it produces a markedly different structure of GPCR oligomer. The full atom model of oligomeric organization resulting from the TM4–TM4 interface was also built for Rh [30]. It was found that the distance between Rh monomers in a dimer was 3.5 nm, and between adjacent dimers 4.5 nm. Double rows were located every 7.7 nm. So this model was not compatible with constraints obtained from AFM data of Rh from native membranes of rod outer segments.

3. Experimental verification of Rh intradimeric interface

Kota *et al* [26] and Guo *et al* [25], using cysteine mutants for Rh and D_2 receptors, respectively, confirmed that both helices TM4 and TM5 are involved in the formation of the intradimeric interface. The residues found to participate in the interface are shown in figure 2. W175 located on the extracellular loop between TM4 and TM5, and Y206 located on TM5 were found to form the interface in the case of human Rh. The other residues shown in figure 2 that were proved to form the interface were found for the D_2 receptor. Residue Y206 (Y5.41 in the general numbering scheme based on the most conserved residue on each TM having the number 50) was confirmed in both studies. Thirteen residues on TM4 were found to crosslink in the D_2 receptor. They were divided into three classes depending on their location on TM4. The first set is located in the central encircled area in figure 2. These residues are on the face of TM4 predicted by using AFM geometric constraints (TM4–TM5 interface). The second set of residues is indicated by the middle dashed ellipse (three residues including W4.50—the most conserved amino acid residue on TM4). The third set (located in the upper dashed ellipse in figure 2) consists of nearly all residues starting from residue 4.56 to 4.62. These residues occupy a highly movable part of TM4 consisting of one turn of α-helix, tilted to the rest of TM4. The wide black arrow in figure 2 indicates the beginning of this area on TM4. The three sets of crosslinking residues are additionally separately encircled in figure 2.

The residue A4.58 was additionally found to crosslink with modified retinal upon Rh activation [31]. This would suggest inward location of this residue but it also crosslinks with A4.58 from another Rh molecule so it also sticks out from Rh. The most probable explanation is that the whole area that A4.58 is located in is unfolded or highly movable, so any direction can be taken. Another situation exists for two other residues (4.50 and 4.54) belonging to TM4–TM4 Rh interface and located on the rigid part of TM4. They also crosslink with the same residues from the second Rh molecule, so it was proposed that TM4 could rotate or the whole receptor could move to change the intradimeric interface [25]. However, three other residues from the cytoplasmic part of TM4 from the TM4–TM4 interface are not crosslinking (4.40, 4.43 and 4.47). So either the rotation of TM4 is confined to the extracellular part of it or the movement of the receptor is connected with a tilt bringing together only extracellular parts of TM4. These possibilities remain unverified. It was clarified, however, that the Rh intradimeric interface is dual.

It was also found that the TM4–TM5 interface is preferred in the presence of inverse agonists (sulpride for D_2) and the TM4–TM4 (at least partly) interface in the case of agonists (dopamine for D_2 receptor) [25]. However, Kota *et al* [26] conducted their crosslinking experiments with opsin (Rh without retinal) and confirmed the TM4–TM5 interface. 11-*cis*-retinal acts as an inverse agonist in Rh preventing the receptor from undergoing activation.

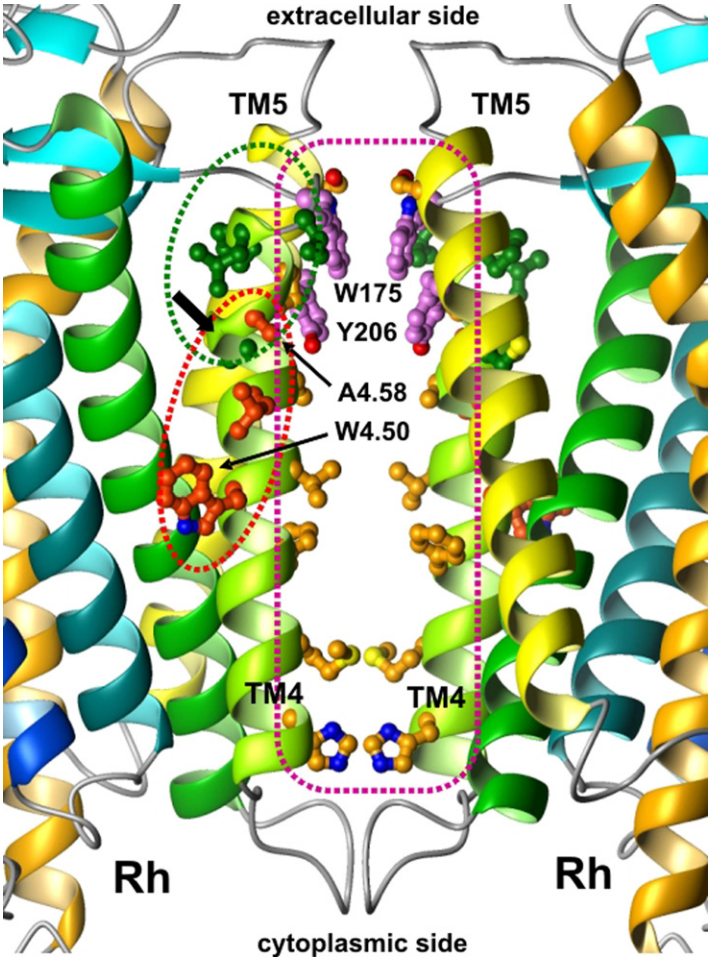

Figure 2. TM4–TM5 Rh intradimeric interface with experimentally proved crosslinking residues. Residues W175 and Y206 were determined for rhodopsin, and the others for the D_2 dopamine receptor. The central encircled area involves residues belonging to the TM4–TM5 interface. The dashed middle ellipse indicates residues belonging to the TM4–TM4 interface. The dashed upper ellipse marks residues on the flexible part of TM4. The wide black arrow indicates the beginning of this flexible area.

Only after Rh bleaching does 11-*cis*-retinal isomerize to all-*trans*-retinal and unbind from Rh, leaving the opsin in the active conformation. So without retinal the opsin should undertake the active conformation which enables it to bind and activate transducin.

4. The Rh–Gt complex

Utilizing the TM4–TM5 interface the complex was built between oligomeric rhodopsin and trimeric transducin [21]. 1N3M (from Protein Data Bank) rhodopsin oligomer was used together with the 1GOT structure of transducin. The C-terminus of transducin (1AQG containing 11-residue fragment) and not seen in the 1GOT structure was fused to Gtα to form a single α-helix. Comparison of the 1N3M model to experimental structures of inactive Rh

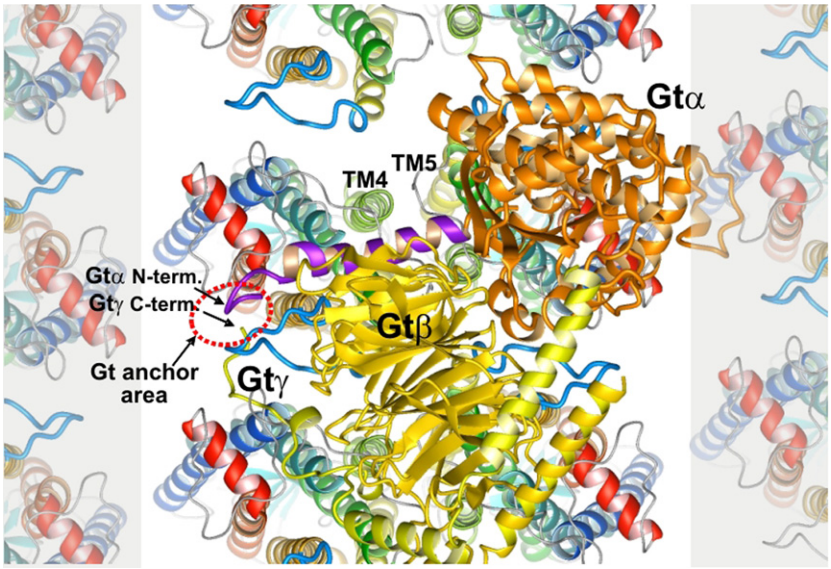

Figure 3. The model of trimeric transducin Gt$\alpha\beta\gamma$ bound to the rhodopsin oligomer with a TM4–TM5 dimeric interface. View from cytoplasmic side. The dashed ellipse marks the Gt membrane anchor area. The Gtα N-terminal helix linking both rhodopsin molecules in the dimer is darker (coloured in purple in the web version). This helix is perpendicular to the TM4–TM5 interface plane in the rhodopsin dimer. In the web version of this article Gtα is coloured in orange, Gtβ in dark yellow, and Gtγ in bright yellow.

molecules was done in [32]. The complex is shown in figure 3 whereas the organization of Rh monomers is the same as in figure 1. The Gtα subunit recognizes the activated Rh (Rh*) by its C-terminal α-helix, whereas the long N-terminal α-helix of Gtα links both Rh molecules in a dimer. It was suggested that this helix is perpendicular to the plane of the TM4–TM5 interface. Only in this position can Gtα bind to a long groove on the cytoplasmic surface of Rh dimer (so-called footprint) that traverses the dimeric interface [21]. Such preorganization of the Rh_2 cytoplasmic surface could greatly reduce the free energy needed for binding of transducin. The location of Gtα determines the positions of Gtβ and Gtγ on Rh oligomer. They are located at, and possibly bind, to adjacent Rh dimer. Adjacent double rows of Rh are unaffected by Gt$\alpha\beta\gamma$ binding. The details of the structure of the Rh_4–Gt$\alpha\beta\gamma$ complex were shown and extensively discussed in [21].

The activated state of rhodopsin (meta II) required for binding to transducin was prepared by manual modification of the transmembrane helix TM6. This movement is assumed to be the main event during rhodopsin activation [33]. The cytoplasmic part of this helix was manually rotated by 90° and moved out of Rh* centre by 0.5 nm. No movements of centres of masses of any rhodopsin molecules in the oligomer were undertaken. This procedure did not affect the oligomeric organization of Rh and in particular the interface TM4–TM5.

The complex was simulated in a membranous environment specific to discs from rod outer segments where Rh is located. The membrane was composed of three types of phospholipid with phosphatidylcholine (PC) headgroups on the extracellular (intradiscal) side and phosphatidylethanolamine (PE) and phosphatidylserine (PS) headgroups (three times more phosphatidylethanolamine headgroups than phosphatidylserine) on the cytoplasmic side [34, 35]. All three types of phospholipid contain the saturated stearoyl chain (18:0) in the *sn*1 position and the polyunsaturated docosahexaenoyl chain (22:6$n-3$) in the *sn*2 position.

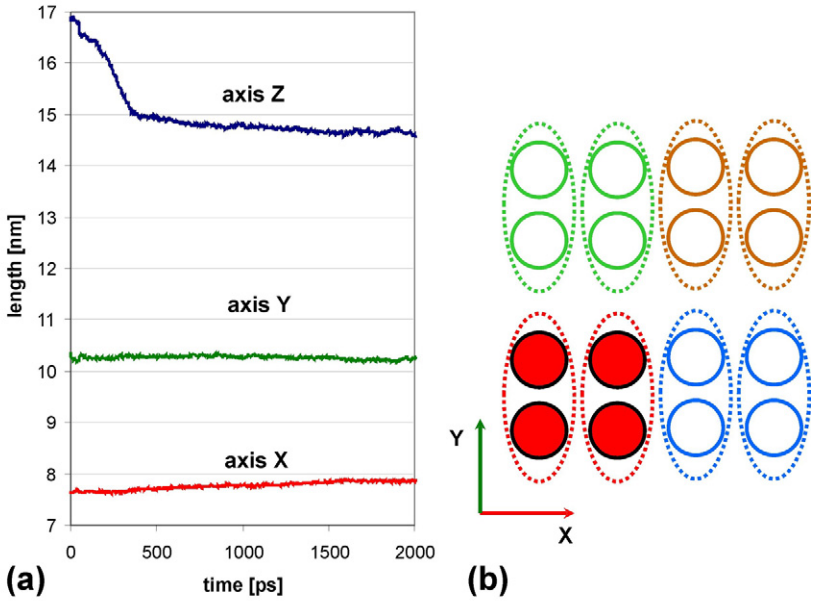

Figure 4. Periodic cell of simulated system. (a) Change of dimensions of periodic cell during simulation (b) Schematic organization of the periodic box and its adjacent images. A real box is shown with filled circles denoting Rh molecules. Dashed ellipses denote Rh dimers. Image cells are indicated with empty circles and different colours.

Because there was three times more PE than PS, the negatively charged PS lipids were inserted close to areas rich in positively charged amino acids, mainly the long cytoplasmic loop between helices TM5 and TM6. In the larger open areas of lipids the PS were evenly distributed. The mobility of lipids in the high oligomeric structure of rhodopsin is greatly reduced and requires manual intervention in PS distribution.

The molecular dynamics simulation was conducted in a periodic box containing two Rh dimers and one Gt trimer. Both Rh and Gt were modified prior to simulation by adding hydrophobic chains. Two palmitoyl groups were coupled with cysteines C322 and C323 just after helix H8 of Rh. The myristoyl chain was coupled to the amino-terminal glycine residue of Gtα, and farnesyl to the C-terminus of Gtγ to a residue C71. The C-terminal part of Gtγ is not seen in the crystal so it was built in extended conformation allowing farnesyl to be in close proximity of myristoyl because they both form a single anchor that attaches Gt trimer to the membrane.

MD simulations were done with the NAMD2 program [36] using an all-atom CHARMM27 force field [37–41]. All simulations were performed in periodic conditions with full electrostatics calculations using the particle mesh Ewald procedure [42] for treatment of the long-range electrostatic interactions (the number of grid points was 80 in the X dimension, 108 in the Y dimension, and 180 in the Z dimension). The size of the periodic box after the equilibration phase was 7.8 nm $\times$ 10.3 nm $\times$ 14.7 nm, and angles $\alpha = 90°$, $\beta = 90°$ and $\gamma = 85°$. All dimensions were stable (figure 4(a)). The schematic organization of the periodic box and its adjacent images are shown in figure 4(b). The cutoff used for calculation of nonbonded van der Waals interactions was 1.2 nm. This guarantees that the Rh molecule and its image do not see each other (the minimal distance between a real Rh molecule and its image was 7.6 nm). The periodic box contained about 150 phospholipids and about 122 000 atoms.

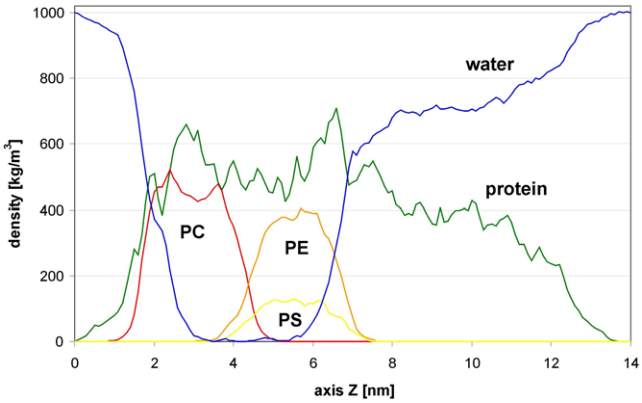

Figure 5. Distribution profiles of water, lipids and proteins (rhodopsin and G protein). Different types of lipids are indicated with labels: PC—phosphatidylcholine, PE—phosphatidylethanolamine, and PS—phosphatidylserine headgroups.

This was maximal number of lipids that can fit within the existing oligomeric network of Rh (figures 1 and 4(b)). TIP3P-type water molecules were used. The system was first equilibrated for 2 ns at 300 K and 1013 hPa with all proteins frozen. This procedure allowed lipids to adjust around the protein and water to diffuse into crevices of the protein and hydrophilic parts of the membrane. This resulted mainly in diminishing of the Z dimension of periodic box (figure 4(a)). Then the system was simulated for an additional 2 ns without any constraints with a constant pressure of 1013 hPa and a temperature of 300 K. The simulation time step was 1 fs.

The obtained distribution profiles of water, lipids and proteins (rhodopsin and G protein) are shown in figure 5. PC lipids are observed at the extracellular side, and PE and PS at the cytoplasmic side. The protein profile is strongly biased towards the cytoplasmic side because of the large trimeric G protein. The profile of water shows some traces of water even in the centre of the membrane but these are single water molecules inside rhodopsins.

The root mean square deviations (RMSDs) of proteins from their initial positions are shown on plots in figure 6(a). Surprisingly, Rh* (bound to the C-terminus of Gtα) was characterized with the lowest RMSD which stabilized at 0.18 nm. Possibly the greater contact with Gt stabilizes the Rh* motion. The RMSD of inactive Rh (holding N-terminal helix of Gtα) stabilized at 0.20 nm. The whole G protein RMSD reached a level of 0.25 nm, very close to the RMSD of all proteins in the complex (four Rh molecules and trimer of Gt). The RMSD plots for Gt and for all proteins are very similar in shape, suggesting that movements of Gt dominated over all rhodopsin molecules. The RMSD of 0.28 nm for the whole complex is a small value, and it indicates that the complex is very stable in the timescale used.

The RMSD plot for lipids is shown in figure 6(b). Lipids are much more mobile than proteins, but as is seen from the plot they occupy the same area mostly because of protein crowding. Their mobility is about one order of magnitude larger than proteins and quite distinct from them. The lipid movements are mostly large and separated by periods of calm. Such movements occur mainly when two lipid hydrophobic chains are able to swap.

5. Membranous anchor area of Gt

Both hydrophobic modifications of Gt continued to stay together during the whole simulation and they preferred to have extended conformations of their chains allowing for extensive contacts with adjacent phospholipids (figures 7(a) and (b)). They are located at the outer

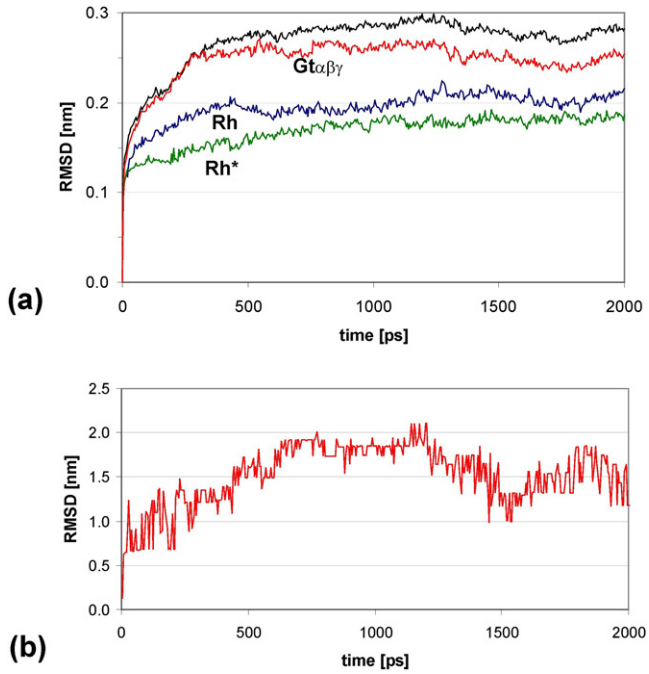

Figure 6. Root mean square deviation plots (a) for particular components of Rh–Gt complex. From the bottom: first line—activated Rh (Rh*) bound to C-terminus of Gtα, second line—inactive Rh bound to N-terminal helix of Gtα, third line—Gt$\alpha\beta\gamma$, highest line—all proteins in the complex. (b) RMSD plot for lipids.

side of a double row (figure 3), which enables easy translocation of transducin along this row and probing for the activated state of Rh. Interestingly, hydrophobic modifications of Rh are located very close to the proposed localization of the Gt anchor area (figures 7(a) and (b)). Because they contain no double bonds they organize the surrounding area by reducing the mobility and thus stabilizing it. In our model, one of the palmitoyl chains became part of the solvation shell of the Gt anchor domain (figure 7(b)). On the average this domain is surrounded by eight phospholipids, and each of them exposes one lipid chain to interact with the Gt anchor domain. It was experimentally determined that Gt$\alpha\beta\gamma$ is tightly interacting with 3 ± 1 phospholipids [43]. Assuming that they interact with Gt with their two lipid chains this result agrees with our simulation. We did not establish preferences of the Gt anchor domain for different phospholipids.

During activation of Gt the farnesyl group is translocated to the interior of the Gtβ subunit and a C-terminus of Gtγ undergoes a conformational switch. The C-terminal region (residues 60–71) changes from an unstructured chain to an amphiphilic helix [44, 45]. Having no membrane anchor and being decoupled from Gtα after activation by Rh*, the Gt$\beta\gamma$ part dissociates and becomes soluble. Gtα also unbinds from the cytoplasmic part of rhodopsin surface allowing Rh* to activate another trimer of Gt. Such events last too long to be directly simulated but still it will be possible to reveal and characterize the landmark structures emerging during the process of Rh–Gt coupling, activation and decoupling.

6. Conclusions

Rhodopsin (Rh) serves as a template for other G protein coupled receptors (GPCRs). The same occurs for rhodopsin G protein (transducin) and recently the dimeric state of Rh proved

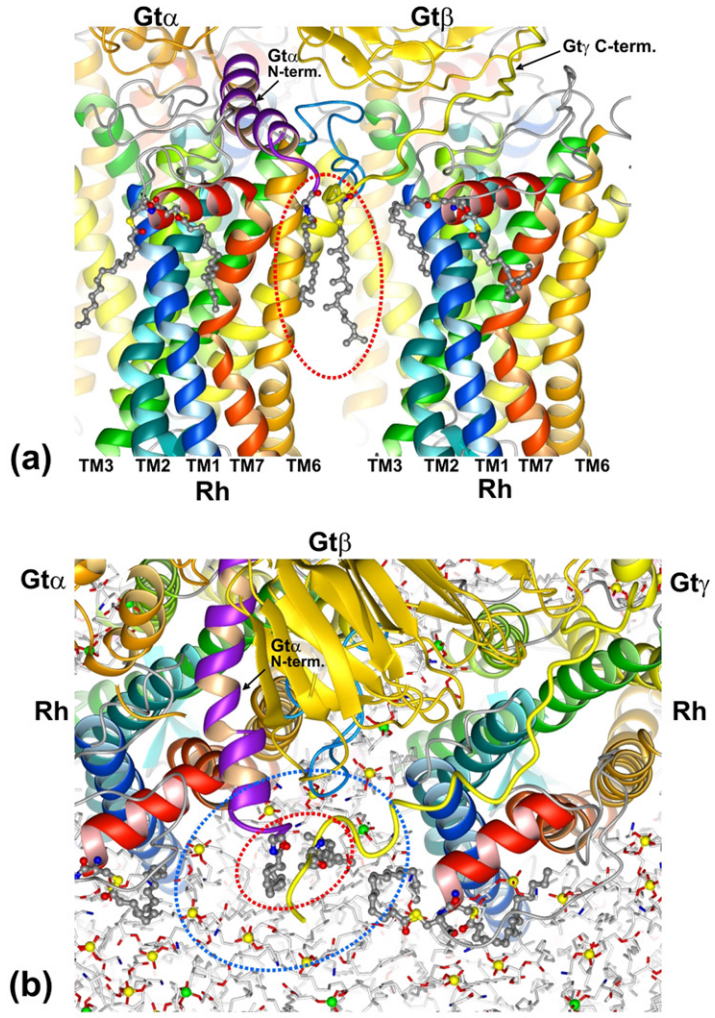

Figure 7. Transducin membrane anchor area (marked by dashed ellipse) being in complex with Rh oligomer. The Gtα N-terminal helix is coloured in purple (in the web version). The loop between TM5 and TM6 linking adjacent Rh dimers is coloured in blue (in the web version). Hydrophobic modifications of Rh and Gt are shown in ball and stick representation. (a) Side view, (b) cytoplasmic view. Phosphorus atoms of phospholipids are shown as spheres coloured in yellow (PEDS) and green (PSDS). Phospholipids involved in interaction with the Gt membrane anchor area are located in the wider dashed ellipse.

to be useful for determination of oligomeric properties of other GPCRs. Here we analysed the stability of oligomeric Rh and trimeric transducin. The model of the complex was based on the Rh intradimeric interface formed by transmembrane helices TM4 and TM5 that was later experimentally proved. We also compared two other existing models of Rh oligomer organization in the native membrane, the first involving TM1, TM2 and TM3 helices in the dimeric interface and the second the TM4–TM4 Rh_2 interface. Experiments performed on Rh and D_2 receptors revealed that residues on TM4 and TM5 are crosslinking to form a homodimer; thus these helices must constitute an intradimeric interface. The interface is dependent upon ligand binding and we discussed possible rearrangements of the TM4–TM5

interface and how they fit the experimental data. The model of the Rh–Gt complex was simulated in the membrane typical for discs from rod outer segments. The Gt membrane anchor domain was characterized and interactions with adjacent phospholipids were described. The analysed model of the Rh–Gt complex proved to be very stable in the timescale 2 ns. This represents the initial stage of Gt activation by activated Rh and needs further experimental verification.

Acknowledgments

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