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The role of membrane curvature elastic stress for function of rhodopsin-like G protein-coupled receptors

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Abstract

The human genome encodes about 800 different G protein-coupled receptors (GPCR). They are key molecules in signal transduction pathways that transmit signals of a variety of ligands such as hormones and neurotransmitters to the cell interior. Upon ligand binding, the receptors undergo structural transitions that either enhance or inhibit transmission of a specific signal to the cell interior. Here we discuss results which indicate that transmission of such signals can be strongly modulated by the composition of the lipid matrix into which GPCR are imbedded. Experimental results have been obtained on rhodopsin, a prototype GPCR whose structure and function is representative for the great majority of GPCR in humans. The data shed light on the importance of curvature elastic stress in the lipid domain for function of GPCR.

1. Introduction

The activation or inhibition of G protein-coupled receptors (GPCR) by ligands is a critical step of cellular signaling in mammals. The ligands of GPCR elicit a diversity of signals via “classical”, G protein mediated signaling pathways [[1], “non-classical”, mostly β -arrestin mediated pathways [2] and, after internalization, via other “non-classical” signaling, recycling or degradation of GPCR [3].

The GPCR are flexible molecules, undergoing rapid thermal motions in their lipidic microenvironment. They exist as an ensemble of conformations in equilibrium. The thermodynamically most favorable conformations predominate in the ensemble. The equilibrium between conformers is primarily shifted by ligand binding but also other mechanisms [4, 5]. Thanks to recent structural studies, insights into structural changes that take place upon receptor activation have been obtained. The GPCR undergo a series of step-wise conformational changes involving functional microdomains within the receptor. Activation has been linked to a rearrangement of transmembrane helices and loops at the ligand binding site, at the N-terminal side of the receptor near the cell surface. Those local structural changes are then amplified and transmitted to the downstream effector binding sites on the C-terminal face of the receptor inside cells [6–8].

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The principal features of conformational changes upon receptor activation appear to be similar for all rhodopsin-like GPCR that have been studied so far [9]. They involve an outward movement of helices V and VI, connected by the third intracellular loop, i.e. a change in the shape of the protein. This structural change opens up the intracellular side of the receptor for interaction with G proteins.

The structural changes depend not only on intrinsic energetic changes within the GPCR, but also on elastic deformations within the surrounding lipid domain to which the protein is linked via lipid-protein interaction. The effects of the membrane environment are strongly dependent on lipid composition and hence can vary between different cell types, and also within different regions of the plasma membrane of the same cell. The aim of this review is to summarize data obtained on the prototypical class-A GPCR, rhodopsin, and to provide a mechanistic interpretation of shifts in the degree of receptor activation. The focus will be on the energetic coupling between rhodopsin conformational changes upon activation and elastic membrane deformation, with special emphasis on the influence of membrane curvature elastic stress.

1.1. Rhodopsin as a model GPCR for studying the influence of the lipid microenvironment

Rhodopsin, the dim-light photoreceptor present in the rod cells of the retina, is both a retinal-binding protein and a G protein-coupled receptor (GPCR) [10]. Rhodopsin consists of the apoprotein opsin and the chromophore 11-cis retinal covalently bound by a protonated Schiff base to Lys 296 in transmembrane helix VII. The 11-cis retinal acts in the dark as a strong inverse agonist that constrains rhodopsin in the inactive conformation, ensuring negligible basal activity of the receptor. Absorption of a photon triggers in situ isomerization of 11-cis retinal into all-trans retinal, an agonist of moderate strength, and leads to the formation of a G protein dependent equilibrium between metarhodopsin I (MI) and several distinct metarhodopsin II states (MII). MI formation occurs within a few microseconds and involves a series of fast transformations mainly occurring near the retinal binding pocket such that the global conformation of MI remains very similar to dark-adapted rhodopsin [11]. The MI photointermediate is not G protein binding competent.

In contrast, MII formation takes place on the timescale of milliseconds and is characterized by larger conformational changes taking place outside the protein photochemical core. Three sequential events have been identified along the activation pathway (i) deprotonation of the retinal Schiff base and protonation of its complex counterion to form Meta IIa, (ii) an outward tilt of transmembrane helix VI which forms Meta-IIb, and (iii) proton uptake by a glutamic acid residue in the microdomain that forms the ionic lock between transmembrane helices III and VI to form an energetically stable Meta-IIbH⁺ photointermediate [12–14]. Those structural changes alter the cytoplasmic receptor surface to allow high rates of catalytic nucleotide exchange after binding of the G protein transducin (G_t) [15–17]. Meta-IIb and Meta-IIbH⁺ activates multiple copies of the membrane bound G_t in succession, setting off a biochemical amplification cascade that, in vivo, ultimately results in the signaling of second order neurons and the visual signal. In rod outer segments, the reaction ends with rhodopsin phosphorylation by rhodopsin kinases that allow arrestin binding and prevent further activation of G_t.

1.2. Rhodopsin as a prototype of the majority of GPCR

Why is rhodopsin a relevant model for studying the effect of the membrane environment and what are the advantages of conducting experiments with this GPCR?

- The overall conformational changes upon activation appear to be similar for all GPCR of class A which comprise about 80% of all GPCR in the human genome, with some differences that are specific for each receptor.
- In contrast to most GPCR that require recombinant expression to study their structure and function, dark adapted rhodopsin can be prepared in large quantities from rod outer segments of the retina, is intrinsically thermally stable, and can be studied in a native form including proper post translational modifications. Furthermore, dark adapted rhodopsin is easily reconstituted into bilayers of various, controlled composition [18].
- Rhodopsin can be synchronously activated by a pulse of light allowing simultaneous analysis of a homogeneous population of receptors which is a great advantage for functional and structural studies.
- The photointermediates of rhodopsin are conveniently characterized by UV-visible-, Fourier transform infrared- (FTIR), and electron paramagnetic resonance (EPR) spectroscopy [15, 19–21].
- All-trans retinal is a partial agonist that makes the equilibrium of photointermediates very sensitive to a variety of cofactors including the composition of the lipid matrix [22]. For the reasons above, rhodopsin is an excellent model for elucidating the relationship between membrane lipid composition and constitutive activity of GPCR or activation of GPCR by partial agonists.

2. Curvature Stress

Curvature elastic stress in lipid bilayers is related to the intrinsic propensity of lipid monolayers to curl into a stress-free state. This tendency is particularly strong for lipids with small polar headgroups such as phosphatidylethanolamine (PE) and polyunsaturated hydrocarbon chains such as the six-fold unsaturated docosahexaenoic acid (DHA, 22:6_{n-3}) a polyunsaturated fatty acid found at high concentrations in retinal and synaptosomal membranes. (insert Fig. 1 here)

2.1. Coupling between membrane curvature stress and rhodopsin conformational transitions

In the following, the influence of lipid composition on the MI-MII equilibrium defined as $K_{eq} = [MII]/[MI]$ will be described based on data obtained by UV-visible spectroscopy. Assuming a rapid exchange between MI and MII photointermediates, the equilibrium of states is described by a Boltzmann distribution, yielding $G \propto \ln(K_{eq})$, where G is the difference in free energy between the photointermediates MI and MII. This difference in free energy not only reflects the changes of intrinsic energy of the protein, but also differences of elastic deformation of the lipid matrix induced by the protein.

The deformation of the lipid matrix by rhodopsin is detected by ^2H NMR order parameter studies on lipids with perdeuterated *sn*-1 hydrocarbon chains [23]. The order parameters report the effective orientation of methylene groups of hydrocarbon chains with respect to the normal to lipid bilayers, time averaged over 10 microseconds. Average order parameters of saturated *sn*-1 hydrocarbon chains have been linked quantitatively to the hydrophobic thickness of bilayers and lateral area per lipid molecule.

Furthermore, lipid hydrocarbon chains in fluid bilayers have high order in the first half of the chain from the carbonyl group to the middle of the chain and rapidly declining order in the second half of the chain towards the terminal methyl group. This feature is commonly referred to as the order parameter profile. Relative changes of order parameters along the chain, or in other words, a change of shape of the order profile is a qualitative reflection of membrane curvature elastic stress.

2.3. Hydrophobic matching between transmembrane helices of GPCR and the lipid matrix

One of the basic principles of membrane biophysics is that the length of hydrophobic stretches on transmembrane helices and the hydrophobic thickness of lipid bilayers must match [24]. Otherwise, hydrophobic residues on proteins and lipids would be exposed to polar residues and water which is energetically highly unfavorable. In reality, both the protein adjusts to hydrophobic thickness of bilayers by adjusting length and tilt of transmembrane helices and the lipid hydrocarbon chains of bilayers stretch or compress to adjust to the length of transmembrane helices [25]. Adjustment of thickness in lipid bilayers generates curvature in the two monolayers of the bilayer.

The latter is easily detected by lipid order parameter studies. In experiments on rhodopsin reconstituted into phosphatidylcholines (PC) with hydrocarbon chain length from 14 to 20 carbon atoms, we established that incorporation of rhodopsin caused the least perturbation of average membrane order parameters if the membranes had a hydrophobic thickness of 27 ± 1 Å. It is equivalent to PC membranes with a chain length between 16 and 18 carbons per hydrocarbon chain. Interestingly, this corresponds to within one Angstrom to the hydrophobic thickness of synaptosomal and retinal membranes. It strongly suggests that GPCR are designed such that perturbation of the lipid matrix from their incorporation is minimal.

2.4. Curvature stress and the MI/MII equilibrium

The contribution from membrane deformation to the energetics of the MI/MII equilibrium has a profound influence on rhodopsin function. With increasing hydrophobic thickness of lipid bilayers, the curvature of monolayers near the protein changes from negative (headgroup area smaller than the chain area) in thin bilayers like 14:0-14:1PC, to neutral, to positive (headgroup area larger than chain area), like for 20:0-20:1-PC. The transition from MI to MII increases stresses in bilayers that are too thin and decreases stresses in bilayers that are too thick. Consequently, the amount of MII formed should increase with increasing bilayer thickness, which is exactly what is observed experimentally.

This raises the question if the increase in the amount of MII formed can be predicted quantitatively by a calculation of differences in energy of membrane perturbations between lipid domains surrounding the MI and MII states. It was shown earlier [26, 27], that in a crude approximation, the differences in lipid perturbation, G_L , can be estimated by the formula

$$\Delta G_L = \frac{A_L \cdot n_L}{k \cdot T} [k_c^{mono} (1/r_{MII} - 1/r_{MI}) \cdot 1/r_0 + const],$$

where A_{lip} is the lateral area per lipid molecule, n_L the number of lipids surrounding the protein, k the Boltzmann constant, T the absolute temperature, k_c^{mono} the bending elastic modulus of the lipid monolayer, and r_{MII} and r_{MI} the effective radii of monolayer curvature of lipid monolayers near the MI and MII photointermediates, respectively, and r_0 the spontaneous radius of curvature of a stress free lipid monolayer.

For lipid bilayers that match the hydrophobic length of transmembrane helices of rhodopsin, the difference between internal energies of MI and MII is then proportional to $A_L k_c^{mono} 1/r_0$. These parameters determine the energy of bending elastic deformation. They were determined experimentally for a series of typical lipid monolayers. A comparison of calculated differences in curvature packing stress between MI and MII lipid domains for common lipids is shown in Fig. 3.

The agreement between predicted shifts from changes in curvature elastic parameters and the measured shifts is remarkable, demonstrating that curvature elastic stress in the lipid domain surrounding rhodopsin is the dominant feature that shifts the MI/MII equilibrium [28].

2.5. Cholesterol effects

The lipid monolayers of retinal and synaptosomal membranes contain up to 30 mol% cholesterol, a molecule that is known to increase bilayer thickness, seen as increase of chain order parameters, and to alter membrane elastic properties. Furthermore, at the proper concentration, cholesterol may facilitate lateral segregation of lipids into cholesterol depleted and enriched regions, such as lipid domains or clusters (also called “rafts”). It has long been suspected that GPCR may have enhanced affinity for cholesterol-rich regions which may facilitate GPCR oligomerization.

In experiments on PCs with various chain lengths, cholesterol concentrations in the range from 0–30 mol%, and rhodopsin concentrations from 0–1,000 lipids per rhodopsin, we observed the following:

- Membranes with a hydrophobic thickness of less than 27 Å favor MII formation upon addition of cholesterol.
- Membranes with a hydrophobic thickness of 27 Å favor MI upon addition of up to 15 mol% cholesterol. Cholesterol concentrations in the range from 15–30 mol% favor MII.

- Membranes with a hydrophobic thickness of more than 27 Å favor MI upon cholesterol addition.
- While the increase in the amount of MII formed in thinner bilayers seems to be primarily driven by the increase in bilayer thickness, the biphasic behavior for bilayers that match the hydrophobic length of rhodopsin and the increase in MI for thicker bilayers seem to be entirely driven by cholesterol-induced rhodopsin oligomerization. This was confirmed in order parameter studies on the lipid matrix conducted as a function of rhodopsin concentration. Those experiments also established that rhodopsin in bilayers that do not match the hydrophobic length of the protein show an increasing tendency to trigger rhodopsin oligomerization with increasing hydrophobic mismatch. In all instances, rhodopsin oligomerization increased the fraction of MI, the photointermediate that does not activate G protein.

3. Conclusions

The data acquired by us and other laboratories show a remarkable dependence of rhodopsin activation on curvature elastic stress in lipid monolayers. When the hydrophobic thickness of bilayers matches the length of transmembrane helices of rhodopsin, the correlation between calculated differences in curvature stress between lipid domains surrounding the photointermediates MI and MII is sufficiently accurate to quantitatively predict the MI/MII ratio with good precision. This is also validation for curvature elastic properties of lipid monolayers that were experimentally determined on inverse hexagonal phases formed with those lipids, in absence of any protein.

Furthermore, the data revealed that any significant hydrophobic mismatch between the GPCR and the lipid bilayer triggers oligomerization of the protein at protein concentrations that are significantly lower than physiological. Last but not least, for bilayers that match to the GPCR, cholesterol is capable of triggering GPCR oligomerization with a complex dependence on cholesterol concentration in the membrane. Remarkably, in all instances oligomerization favors formation of a GPCR conformer that is not capable of activating G protein.

The role of homo- and heterooligomerization of GPCR in G protein activation has been hotly debated. While we may not exclude that the observed decrease in the capability to activate G protein from homooligomerization is specific for reconstituted GPCR, the data strongly suggest that GPCR monomers are fully capable of activating G protein at high efficiency.

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Highlights

- signal transduction by rhodopsin-like GPCR is modulated by the lipid matrix
- the primary mechanism has been identified as curvature elastic stress
- elastic stress in the lipid matrix also modulates receptor oligomerization
- phosphatidylethanolamines with polyunsaturated hydrocarbon chains greatly enhance receptor activation

Figure 1.

Membrane curvature elastic stress: (1) Lipid monolayers may bend to accommodate lipids with, e.g. smaller headgroups and wider hydrocarbon chains such as 18:0–22:6_{n-3}PE. (2) In a bent conformation, those monolayers have lowest energy. (3) When lipid monolayers form a bilayer, i.e a flat sheet, their ability to bend is limited by the opposing lipid monolayer. Monolayers that have lowest energy when curved are now elastically deformed to be flat — they are under curvature elastic stress [29].

Figure 2.

Bilayer-protein hydrophobic mismatch. (A) Non polar residues are shown in dark grey, polar residues in light grey. When the protein is in conformation I, the protein hydrophobic length of transmembrane helices matches the hydrophobic thickness of the unperturbed bilayer. When the protein is in conformation II, the tilt of transmembrane helices is changing which introduces curvature in lipid monolayers near the protein. Furthermore, the protein hydrophobic length of transmembrane helices may increase. Adjustment to the protein's hydrophobic length induces curvature in lipid monolayers as well. The elastic deformation of the bilayer that differs between states I and II depends on bilayer properties. It contributes to the difference in free energies between states I and II. (B) Order parameter profile of perdeuterated *sn-1* chains of 16:0_{d31}-16:1 PC with (open triangles) and without rhodopsin (filled squares). In the presence of rhodopsin, the acyl chains slightly stretch to match the hydrophobic length of the protein's transmembrane segments. This is reflected as an increase of order parameters in the presence of rhodopsin. Lipid order was calculated from experimental data assuming that monolayer deformation occurs only in the first layer of lipids surrounding the protein.

Fig. 3.

Calculated (grey bars) differences between lipid-protein domain energies, $\Delta(\Delta G)$, from differences in curvature elastic parameters of lipid monolayers. The crosshatched bars show the measured differences in $\Delta(\Delta G) \propto \ln(K_{eq})$, from shifts in the MI/MII equilibrium. For simplicity, the $\Delta(\Delta G)$ of rhodopsin in POPC bilayers was assumed to be zero. POPC – 16:0–18:1_{n-9}PC; DOPC – 18:1_{n-9}–18:1_{n-9}PC, DOPE/Me₂/Me₁ – 18:1_{n-9}–18:1_{n-9}PE/Me₂/Me₁ (PE – NH₃; PE-Me₂-NH(CH₃)₂; PE-Me₁ – NH₂CH₃), SDPE – 18:0–22:6_{n-3}PE; SDPA-PE – 18:0–22:6_{n-6}PE.

Figure 4.

Coupling between bilayer curvature stress and rhodopsin function. (1) Rhodopsin packs at the lowest membrane perturbation in bilayers of hydrophobic thickness of 27 ± 1 Å. Rhodopsin stays monomeric even at the physiological packing density (~ 70 lipids/rhodopsin). MII is favored in bilayers with increasing negative elastic curvature stress (+PE) suggesting that MII conformation supports a release of curvature stress. (2) In bilayers thinner than 27 Å, monolayers deform to minimize the hydrophobic mismatch. MII formation is disfavored by increasing curvature stress. Increasing packing density promotes rhodopsin-rhodopsin interactions, which hinder MII formation. (3) When the bilayer thickness is larger than rhodopsin hydrophobic thickness, MII formation relieves a fraction of the stress stored in the membrane. The likelihood of rhodopsin-rhodopsin interactions increases with increasing packing density. (4). Cholesterol raises bilayer thickness and shifts the energetics of the MI-MII equilibrium. When cholesterol is added to a thin bilayer, curvature stress decreases upon MII formation. On the contrary, when bilayers are thicker than rhodopsin, addition of cholesterol increases the mismatch while raising the elastic stress, promoting rhodopsin oligomerization.