Position of Transmembrane Helix 6 Determines Receptor G Protein Coupling Specificity

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Supporting Information

ABSTRACT: G protein coupled receptors (GPCRs) transmit extracellular signals into the cell by binding and activating different intracellular signaling proteins, such as G proteins (Gαβγ, families Gi, Gs, Gq, G12/13) or arrestins. To address the issue of Gs vs Gi coupling specificity, we carried out molecular dynamics simulations of lipid-embedded active β2-adrenoceptor (β2AR*) in complex with C-terminal peptides derived from the key interaction site of Ga (GaCT) as surrogate of Gαβγ. We find that GaCT and GsαCT exploit distinct cytoplasmic receptor conformations that coexist in the uncomplexed β2AR*. The slim GaCT stabilizes a β2AR* conformation, not accessible to the bulkier GsαCT, which requires a larger TM6 outward tilt for binding. Our results suggest that the TM6 conformational heterogeneity regulates the catalytic activity of β2AR* toward Gi or Gs.

G protein coupled receptors (GPCRs) transduce a large variety of extracellular signals into the cell. One and the same receptor can activate different intracellular downstream signaling proteins such as heterotrimeric G proteins (Gαβγ, families Gi, Gs, Gq, G12/13) or arrestins (arrestin 1–4), resulting in different cellular and physiological responses.1 Understanding the molecular mechanism of this coupling promiscuity is therefore one of the key questions in current receptor research. The phenomenon that ligands can bias toward arrestin- or G protein-specific signaling, respectively, has been linked to the existence of different conformations of the active receptor (R*), with different G protein and arrestin binding properties.2 The first structural evidence for how different ligands bias the human β2-adrenoceptor (β2AR) toward arrestin or G protein signaling has recently been provided.3 However, little is known about the structural mechanism by which receptors select between different G protein signaling pathways.4

In this study we performed classical all-atom molecular dynamics (MD) simulations to investigate how the conformational space of the active human β2AR* is exploited for selective coupling to Gs or Gi. For comparison we chose bovine rhodopsin (RhR), which is capable of interaction with only a single G protein, namely Gt, a member of the Gi family. Our report will focus on the conformational heterogeneity of transmembrane helix 6 (TM6), which is released from the 7-TM helix bundle upon activation, resulting in the prominent outward tilt5 observed in crystal structures of active GPCRs.6–9 This hallmark event of GPCR activation leads to the formation of a highly flexible cytoplasmic crevice10–12 to which the C-terminus of the Ga subunit (GaCT),6,7,13,14 a key determinant of G protein coupling specificity,15 binds. Our present analysis suggests that distinct TM6 outward tilts are responsible for Gi- and Gs-specific signaling by β2AR*.

So far, the only crystal structure of a R*-G complex reported is that of β2AR* bound to Gs.7 Comparison with crystal structures of active rhodopsin (RhsR) in complex with GtRCT6,16,17 reveals that both Ga C-termini adopt an α-helical conformation terminated by a reverse turn. GsαCT and GtRCT bind with the tip of the reverse turn to R* (Ballesteros–Weinstein nomenclature) from the E(D)RY motif at the base of the cytoplasmic crevice of R*. However, the reverse turn of GsαCT is bulkier than those of GtRCT and its close homologue GaCT (Figure S9). Specifically, the cation–π interaction between Y391 and R1315,16 in the β2AR*-Gs complex seems to require a 5–6 Å larger TM6 outward tilt than the hydrogen bond between the carbonyl oxygen of C347 and the guanidinium group of R1355,16 in RhR*–GtRCT (Figure 1A,B). The present study was thus motivated by the idea that the different space requirements for the key interactions of GtRCT and GsαCT with R* result in distinct TM6 outward tilts and differently shaped cytoplasmic crevices in the corresponding complexes (Figure 1C,D).

To find out whether a RhR*-like conformation exists in β2AR*, which might be stabilized by GtRCT and thus be responsible for selective signaling through Gi, a series of MD simulations of the uncomplexed β2AR* was started using the receptor coordinates from the β2AR*-Gs complex.7 After in
In accordance with earlier analyses,10 independent 200 ns MD simulations were carried out. (A–C) Side view: The outward-tilted TM6 of βAR* allocates space for the bulky GsCT (outlined, transparent surface) that would clash (indicated by the red outline in C) with the more inward-tilted TM6 position of RhR* (orange) transparent surface) that would clash (indicated by the red outline in C) with the more inward-tilted TM6 position of RhR* (orange) in complex with GsCT. R3.50 from the base of the cytoplasmic crevice and Y391/C347 from the tip of Gs/ταCT are shown as sticks. (D) Schematic representations of the two crystal structure interactions in panels A–C.

Figure 2. Conformations of the cytoplasmic crevice of βAR* observed in MD simulations (A,D) alone, (B,E) with GsCT19, and (C,F) with GiCT19. Panels A–C show the time traces of the TM6 tilts (δTM6−6) with resulting distributions and observed probabilities p. Panels D–F schematically depict the Gs coupled state (blue) and the RhR*–GsCT state (orange). In 3 of 21 simulations (100–600 ns), βAR* adopts a RhR*-like conformation with GsCT19 crystal structures of R and R* exhibit the following TM6 tilts: inactive βAR (PDB entry 2RH1), 18.2 Å; βAR*-Gs (3SN6), 29.7 Å; inactive RhR (1U19), 16.4 Å; and RhR*-GsCT (3PQR), 23.2 Å.

Figure 3. Umbrella sampling run along the coordinates of the TM6 inward movement during transition from the starting state using the receptor coordinates from the βAR*-Gs complex to the βAR*–GiCT19 complex (see Figure 2C). (A) PMF energy profile (black line) and error estimation (gray area). Arrows indicate the positions of the initial (Gs coupling state, blue) and the final βAR* conformation (red) on the transition coordinate (see Supporting Information). An energy barrier of 3.0 ± 2.3 kT is at 2.3 nm on the transition coordinate, which corresponds to d2AR−CT ≈ 28 Å. (B) Increase of the βAR* GiCT19 interaction surface accompanying the TM6 inward movement.

Selective stabilization of a specific R* conformation out of an ensemble of substates has been suggested as a common mechanism for GPCR-mediated signal transduction.12,19 In accordance, the βAR*-Gs complex reconstituted in silico into a lipid bilayer remains essentially unchanged during five 200 ns simulations, although stabilizing mutations, nanobodies, and the fused T4-lysozyme have been eliminated. TM6 (Figure S2A) and GsCT (Figure S3C) are locked in their positions, and the cation–π interaction between Y391 and R1313.50 of the receptor persists (Figure S4A). In addition, several other polar and hydrophobic interactions, most of them also present in the crystal structure, fixate GsCT to ICL2 and ICL3 (Figure S5A).

Gs/ταCT peptides have been used as surrogates of G to examine G protein-selective βAR* conformations.15,20 To identify the minimum peptide length required for complex stabilization, we truncated Gs to a GsCT 19-mer (GsCT19, 578FNDCRDI1QRMHLRQ61EL199). GsCT19 (Figure S3) and TM6 (Figures 2B) remain in their conformations during...
all simulations, stabilized by the same specific interactions as with Gs (Figures S4B and S5B). Further N-terminal truncation to GsaCT11 reduces the number of specific contacts with β2AR* (Figures S4C and S5C), leading to a higher positional variability of GsaCT11 (Figure S3) and TM6 (Figure S2). Our simulations suggest that the binding interface for Gs coupling to β2AR* consists of 15 C-terminal residues (see Figures 4A, S4A–C, and S5A–C) which form a scaffold between ICL2 and TM5/6, stabilizing the more open cytoplasmic crevice of the Gs coupled receptor. Notably, in simulations of a putative RhR*-GsCT peptide complex (see SI Methods), the characteristic cation–π interaction between Y391 and R131 takes form as with β2AR*-GsCT (Figure 4A), in accordance with the absence of a Gs signaling pathway in the visual RhR system.11

Subsequently, we addressed the question whether a Gsa C-terminal 19-mer (GsaCT19, 336FDAVTYI1K3NLKD4CGLE550) can stabilize the RhR*-like conformation observed for the uncomplexed β2AR*. The starting position of GsaCT19 was extrapolated from the crystal structure complex of GsaCT19 (355FDAVTYI1K3NLKD4CGLE550) with RhR*.6 The receptor coordinates were taken from the β2AR*-GsCT crystal structure complex.7 In this starting configuration GsaCT19 does not have any contact with TM6. Similar to the simulations of uncomplexed β2AR*, TM6 tends to persist in its starting position within the first hundreds of nanoseconds (Figure S2E). However, in 3 out of 21 independent simulations, the initial receptor conformation is left, and TM6 tilts inward spontaneously by 6 Å (Figure 2C).

To obtain information about the energy barrier that retards formation of the more closed β2AR* state bound to GsaCT (d3M/6 = 23 Å), we performed umbrella sampling (US) MD simulations along one of these trajectories and calculated the potential of mean force (PMF, Figure 3A). The PMFs show that such an energy barrier exists, arising from the reorganization of interactions between TM5/6 (Figure S6B). As soon as this barrier is overcome, the TM6 inward movement follows a continuous downhill reaction. The interaction surface with GsaCT19 that remains at its starting position increases (Figure 3B), and the key interaction between C351 and R131 forms. This key interaction and a hydrogen bond between N347 and P1383.57 from the second intracellular loop (ICL2) and from D341 and K345 to ICL3 are also observed in MD simulations of GsaCT with RhR* (Figures 4C and S5E), supporting the notion that β2AR* forms a complex with GsaCT very similar to the complex RhR* forms with GsaCT (Figure S7C). The α-helical content of TM6 increases upon complex formation (Figure S6A), in accordance with our recently proposed model of receptor G protein coupling through structural stabilization of the binding crevice.11 Extrapolation of the GsaCT19 peptide to the Gi holoprotein in a putative β2AR*-Giαβγ complex (model based on β2AR*-Gs7) results in a feasible arrangement without clashes (Figure S7A). Finally, our analysis of Gi vs Gs interactions explains previous mutational data, in which a chimeric Gsa/Gtα was capable of binding to and being potently activated by RhR*22. The two Gsa triple mutants that made Gsa light-activatable were indeed exchanged at those amino acid positions, where we observe differential interactions of Gs and Gi, namely Y391 to R350, E392 to TM6/H8 for Gs (Figure 4A) or N347 with ICL2, C351 with R350, and D350 with ICL1 for Gi (Figure 4B).
In accordance with earlier studies, we observe a strong structural heterogeneity for the two active GPCRs, β2AR* and RhR*, reflected by broad distributions of different TM6 tilts in the absence of intracellular interaction partners. In the case of β2AR*, this distribution exhibits at least two pronounced maxima at 23 and 28 Å. The maximum at 28 Å represents the Gs coupled state, while the maximum at 23 Å indicates a second β2AR* conformation very similar to the one observed in the GsCT-RhR* complex. GsCT, a close homologue of GsCT, stabilizes more closed β2AR* conformation while due to its bulkiness −CT requires the more open cytoplasmic crevice with a much larger TM6 outward tilt for binding. These observations suggest that Gs and Gi/t have different binding modes, both imprinting their own shape onto the cytoplasmic R* crevice through specific interactions of GsCT with β2AR*.

Our results extend an emerging concept of GPCR signaling based on multiple receptor conformations in equilibrium, each exhibiting specific affinities to the variety of extracellular ligands and intracellular proteins. In the example studied here, the Gα C-termini of Gs or Gi, representing the key sites for interactions with the active β2AR*, are able to select and stabilize specific active conformations from a pre-existing equilibrium of agonist-bound receptor states (Figure 2). Thus, the receptor provides a characteristic amount of TM6 flexibility, while the Gα C-terminus selects a specific conformation for productive interaction and signal transfer by presenting a surprisingly small number of crucial residues (Figure 4 and ref 22). Taken together, the abundance of a specific R* conformation, in addition to the availability and affinity of different G proteins, co-determines which pathway prevails in the intracellular network.

ASSOCIATED CONTENT

Supporting Information
Preparation and execution of MD simulations and US calculations, performed and analyzed with GROMACS; GPCRs were selected on the basis of the availability of an X-ray structure of a receptor G protein (or peptides derived thereof) complex, which is a prerequisite for sufficiently accurate atomistic simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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