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Effect of intracellular loop 3 on intrinsic dynamics of human β_2-adrenergic receptor

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Abstract

Background: To understand the effect of the long intracellular loop 3 (ICL3) on the intrinsic dynamics of human β_2-adrenergic receptor, molecular dynamics (MD) simulations were performed on two different models, both of which were based on the inactive crystal structure in complex with carazolol (after removal of carazolol and T4-lysozyme). In the so-called *loop* model, the ICL3 region that is missing in available crystal structures was modeled as an unstructured loop of 32-residues length, whereas in the *clipped* model, the two open ends were covalently bonded to each other. The latter model without ICL3 was taken as a reference, which has also been commonly used in recent computational studies. Each model was embedded into POPC bilayer membrane with explicit water and subjected to a 1 μs molecular dynamics (MD) simulation at 310 K.

Results: After around 600 ns, the *loop* model started a transition to a "very inactive" conformation, which is characterized by a further movement of the intracellular half of transmembrane helix 6 (TM6) towards the receptor core, and a close packing of ICL3 underneath the membrane completely blocking the G-protein's binding site. Concurrently, the binding site at the extracellular part of the receptor expanded slightly with the Ser207-Asp113 distance increasing to 18 Å from 11 Å, which was further elaborated by docking studies.

Conclusions: The essential dynamics analysis indicated a strong coupling between the extracellular and intracellular parts of the intact receptor, implicating a functional relevance for allosteric regulation. In contrast, no such transition to the "very inactive" state, nor any structural correlation, was observed in the *clipped* model without ICL3. Furthermore, elastic network analysis using different conformers for the *loop* model indicated a consistent picture on the specific ICL3 conformational change being driven by global modes.

Keywords: ICL3, Molecular dynamics simulation, Transmembrane helix 6, G-protein binding site, Ligand docking, Essential dynamics

Background

As the largest family of membrane proteins in the human genome, the G protein coupled receptors (GPCRs) are structurally characterized by the presence of seven membrane-spanning α-helical segments with an extracellular N terminus and an intracellular C terminus. Upon binding to agonists, a series of conformational changes propagate along transmembrane helices and reach the intracellular part of the receptor, which directly interacts with the hetero-trimeric G-protein. Consequently, G protein's activation triggers different cascades of events depending on the type of agonists bound to the receptor. Therefore, as the initiation point to the flow of signals into cells, GPCRs are associated with a plenty of diseases that make members of this family significant pharmacological targets.

The first solved X-ray crystal structure of GPCR belongs to bovine rhodopsin [1,2], which is followed by the crystal structure of human β_2-adrenergic receptor (β_2AR) in the inactive state [3,4]. Since 2007, the cholesterol bound form of β_2AR (PDB:3D4S) [5], the structure of turkey β_1-adrenergic receptor (PDB:2VT4) [6], the structure of a methylated β_2AR (PDB:3KJ6) [7] and various forms of inactive states of β_2AR bound to antagonists such as ICI 118,551 and alprenolol (PDB:3NY8,3NY9,3NYA,3PDS) [8,9] have been reported. Finally, the nanobody-stabilized active state of β_2AR in complex with G-protein, has been solved by

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Rasmussen and his coworkers (PDB:3SN6) [10,11]. Still, these static pictures of the receptor remain insufficient to describe the dynamic character of the receptor, which governs the function. It is a well-established concept that proteins have an intrinsic ability to sample an ensemble of distinct conformations in order to perform certain functions [12]. The ligand simply selects the optimal receptor conformation for binding followed by an induced fit to stabilize the final conformation. Many questions remain on these multiple, ligand-specific conformational states of β_2AR with different levels of activity from fully active to fully inactive, which induce distinct signaling pathways.

The ternary complex model proposed in 1980 by Lefkowitz and his coworkers [13] describes an allosteric mechanism for receptor activation. The agonist molecule, when bound to the extracellular part, simply promotes and stabilizes the high affinity β_2AR-G protein complex. Following the laws of thermodynamics, binding of G-protein increases the receptor's affinity for agonist binding to the same extent. Fluorescence spectroscopic studies of β_2AR by Ghanouni *et al.* [14] presented a model with multiple, agonist-specific receptor states, in which the activation occurs through a sequence of conformational changes. They also suggested that the activation barrier for transition from intermediate to active state is high, and that *in vivo* the barrier is more likely reduced by G protein binding. The presence of an intermediate state is further supported by the fluorescence spectroscopy studies of Swaminath *et al.* [15,16], suggesting a mechanistic model for GPCR activation, where agonist binding stabilizes a series of conformational states with distinct cellular functions.

In addition to experiments, several MD simulation studies have been conducted after the inactive and active states of the receptor have been solved by X-ray crystallography. One simulation study by Dror *et al.* [17] reveals that the receptor exists between two distinct inactive conformations of the receptor, one with the ionic lock intact and one with the lock broken. In 2011, Dror and his coworkers proposed a completely different activation mechanism in which the structural changes start at the G protein binding site propagating upwards as opposed to agonist-induced conformational changes that start at the agonist binding site and propagate down to G protein binding site [18]. The agonist-bound crystal structure of β_2AR without a binding partner (PDB:3PDS) recently revealed by Rosenbaum *et al.* [9] is found to be identical to the inactive state of the receptor (PDB:2RH1). This suggests that in the absence of a G-protein, the receptor prefers to adopt the inactive conformation whether or not it is bound to an agonist. In other words, the agonist molecule is not sufficient alone to shift the equilibrium to the active state. Dror *et al.* [18] also proposed an intermediate state for G-protein binding site, which exists as a part of the receptor's intrinsic dynamics. Binding of a G-protein to this binding site simply promotes a transition to the active conformation, which is further stabilized by an agonist bound at the extracellular region. The most important feature about the dynamics of β_2AR is the strong coupling that exists between the intracellular G-protein binding site and the extracellular ligand-binding site of the receptor [7,19]. The receptor behaves like a pair of pincers where the intracellular part becomes narrower as the extracellular part becomes wider, and vice versa.

Due to its unstructured nature, ICL3 region is either unresolved in crystallographic experiments or completely removed and replaced by T4-lysozyme (T4L) to facilitate the crystallization. Thus, none of the experimental and simulation studies have discussed the possible effect of ICL3 on the intrinsic dynamics of the receptor. Its replacement by T4L to facilitate crystallization did not prevent agonist-induced conformational changes based on fluorescence spectroscopy measurements [20]. Yet, it is well accepted that its direct interaction with G-protein probably have a significant role on the receptor's dynamics and the activation/inactivation pathway [21,22].

In this study, the effect of ICL3 on receptor's conformational dynamics was investigated via two distinct models of the receptor. Both models were generated from the inactive state of the receptor (PDB:2RH1) after removal of T4L. Moreover, the partial inverse-agonist carazolol was removed from the binding site of both models, since the goal of this work was to provide data about the intrinsic dynamics of the receptor, i.e., the ensemble of conformations accessible to its apo form. According to the current view on ligand binding, the equilibrium distribution of conformational states may be shifted upon ligand binding.

In the so-called loop model, the ICL3 region was modeled as an unstructured loop of 32-residues length and inserted between two open ends of TM5 and TM6 (residues 230 and 263), whereas in the second model, these two open ends were "clipped" or simply covalently attached to each other. The "clipped" model of the receptor, serving as a reference in our study, has been commonly used in recent simulation studies as well [18,23]. Both models were subjected to 1 μs MD simulation in a POPC membrane bilayer at 310 K. The essential dynamics analysis was carried out to reveal important allosteric coupling within the receptor in the presence of ICL3. Two distinct snapshots taken from the loop model's trajectory were further used as docking targets for an agonist and an antagonist molecule in order to investigate the effect of ICL3 on binding site conformations. Finally, elastic network analysis was performed on different conformations and loop models to reveal a consistent picture on receptor intrinsic dynamics.

Results and discussion

Presence of ICL3 affects RMSDs and loop mobility

The difference between loop and clipped model dynamics is illustrated in the root mean square deviation