Computational study of $\mu$-opioid receptor embedded in a realistic membrane

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Abstract. Opioids are highly effective analgesics used to alleviate acute, surgical and cancer pains. However, utilization of opioids leads to adverse effects such as nausea, constipation, dizziness, somnolence, vomiting, especially, high addictive liability and drug-induced respiration depression. Ongoing efforts to generate safer opioid analgesics, such as designing biased agonist, peripheral opioids or endogenous opioid analogues, requires thorough understanding of the relationship between structure and function of the main target of opioids - $\mu$ORs. In this research, the structural and dynamic of a $\mu$OR molecule embedded in membrane are studied by molecular dynamics simulation. Our results revealed the influences of disulfide linkage between CYS142-CYS219 and the transmembrane proline and glycine on $\mu$OR’s dynamics and structure, as well as, their important role in $\mu$OR’s functions.

1. Introduction

Every year, millions of pain relief drugs prescriptions are written, and many of them are opioids. Opioids are among the strongest pain relief in clinical use, but their analgesic effect is accompanied with many serious adverse effects, such as constipation, nausea, vomiting, respiratory depression, and addiction. Opioids overdose has been responsible for thousands of deaths every year [1]. These severe issues have been the driving force behind the development new effective painkillers which create less side effects.

Opioids induces analgesic effects mainly by binding to opioid receptors which are members of the super family of G protein coupled receptor (GPCR). Three types of opioid receptor, including mu ($\mu$OR), delta ($\delta$OR) and kappa ($\kappa$OR), are potential targets for drugs related to mood and pain. Among them, $\mu$ORs are the most important receptors in regulating pain relief [2], and thus, are the target of most painkiller design studies. Recently, there are new strategies to develop safer opioid analgesics [3]. The approach attracts most attention is "biased agonism". Like other GPCRs, the most recognized signaling pathways of $\mu$OR are G proteins and $\beta$-arrestins which present different biochemical and physiological functions. After binding $\mu$OR, most of known opioids are thought to equally activate both G protein and $\beta$-arrestins signaling pathways. However, biased agonists are able to selectively activate the signaling pathway leading to the analgesic effects but not the signaling pathway causing constipation, respiratory depression, addiction and other adverse effects [4] [5]. TRV130 is the first biased agonists of $\mu$OR which have demonstrated its improved treatment and safety in clinical trials for acute pain management [6] [7]. In addition, another compound, PZM21, discovered by
computational modeling and structure-based drug design, is biased ligand of µOR, however, recent study found that PZM21 could produce respiratory depression, similarly to morphine. Other approaches are very promising for development of safer painkiller targeting µOR, for example peripheral opioids, allosteric µOR drugs and endogenous opioid analogues [3]. These strategies are appealing, but need further research to obtain the goal of safer painkillers targeting µOR.

To assist in development of better painkiller with less side effects, clearly much more works are needed. Especially, a better understanding of the molecular structure and dynamics of opioid receptors is a must. In this work, we focus on molecular study the stability of µOR in a realistic membrane by molecular dynamics simulation. There are pieces of evidence that the active conformation of GPCR can be stabilized by interactions between the receptor and its Gα protein. Furthermore, C-terminus of the Gα subunit is deeply bound in a pocket between the transmembrane domains. Thus, getting rid of this part of the Gα and substituting by polar water molecules will result in some problems in subsequent molecular dynamics simulations. Therefore, in this work, the full structural model of µOR and Gα will be constructed and studied (Figure 1). We will focus on the interacting picture at molecular level of various residues that are known from experiments to play essential role in opioid binding.

This paper is organized as follows. After the introduction in Section 1, the detail of the computational procedure is presented in Section 2. In Section 3, the results are presented and discussed. We conclude in Section 4.

2. Computational details

2.1. Simulation systems preparation

A model including µOR and Gα (see figure 1a) was set up from the structure of activated µOR in complex with Gi with protein data bank ID - 6DDF [8]. Because Gα in 6DDF.pdb has

Figure 1: The overview structure of the complex: (a)Complex of µOR (red) and Gα protein (blue); (b) mixed bilayer membrane including CHOL (purple), DPPC (green) and DOPE (cyan); (c) the system where µOR was embedded in a well-equilibrated membrane
many missing residues, a full structure of G\(_\alpha\) was created by manually mixing G\(_\alpha\) in 6DDF with G\(_\alpha\) in 3UMS [9]. The original structure of 6DDF was downloaded from OPM website (https://opm.phar.umich.edu/) which aligns the protein in the membrane (in the xy plane) and also center the transmembrane domain in the middle of the membrane. The server PDB2PQR (http://nbcr-222.ucsd.edu/pdb2pqr_2.1.1/) was used to define the protonation state and charge of all titratable residues [10]. The essential disulfide bonding between Cys142 and Cys219[12] was created before simulation.

Membrane lipid composition plays an important role in structure/function relationships of \(\mu\)OR. A bilayer membrane with similar composition to synapse membrane was created by Membuilder server (http://bioinf.modares.ac.ir/software/mb2/). Lipid components chosen for the membrane are cholesterol (CHOL), DOPE and DPPC with the ratio of 6:5:4 respectively. This ratio approximates the lipid composition of synaptic plasma membranes of rat brain [11]. The proteins were embedded into the membrane which was equilibrated by a 50ns molecular dynamics simulation (See Figure 1b). The specific numbers of molecule for the system are listed in Table 1.

| Component | # of molecules |
|-----------|----------------|
| \(\mu\)OR | 1              |
| G\(_\alpha\) protein | 1          |
| CHOL      | 317            |
| DOPE      | 204            |
| DPPC      | 240            |
| Water     | 63731          |
| Na        | 307            |
| Cl        | 319            |

Table 1: The components of the system.

2.2. Molecular dynamics (MD) simulation

All molecular dynamics simulations conducted here were performed using the software package GROMACS 2016.4 [13]. AMBER99SB-ILDN [14], Slipid [15] and TIP3P force fields are used to parameterize the proteins and ions, the lipids and water, respectively. Particle Mesh Ewald method is used for treatment of long-range electrostatic interaction. Semi-isotropic coupling of pressure is chosen to uniformly scale x-y box vectors while independently scale z axis. A cutoff radius of 1.2 nm is set for treating electrostatic and Van der Waals interactions. LINC algorithm is chosen to constrain all bonds involving hydrogen atoms. At first, systems is optimized using steepest descent minimization with a threshold maximum force constant of 1000 kJ/mol/nm. 50 ns of NPT ensemble is then performed at reference temperature of 310K with Nose–Hoover thermostat and reference pressure of 1 atm with Parrinello–Rahman barostat. Finally, 200ns of MD production with the condition similar to NPT is performed for statistical analysis. For sampling, frames are collected every 100 ps.

2.3. Analysis the results of MD simulation

Analyses of the simulation results are performed using the corresponding tools provided within the GROMACS package, such as the root mean square deviation (RMSD) for backbone C\(_\alpha\) atoms of the proteins as well as their root mean square fluctuation (RMSF). The secondary structures of \(\mu\)OR are determined by DSSP. The visualization of 3D structure on the systems are performed using VMD version 1.9.3 program [16].
3. Results and discussion

3.1. Deviation of the protein complex

Figure 2: Time revolution of the RMSD of protein backbone atoms for μOR (a) and Go protein (b) during 200ns simulation

First, we investigate the overall stability of our complex by measuring the deviation of the proteins (Figure 2) structure from its native structure. The backbone root mean square deviation (RMSD) of μOR is stable from 45 ns. Whereas, backbone RMSD of Go protein is much larger and have not shown a plateau. These are easy to understand. μOR is more stable than Go protein because μOR is embedded in the membrane, while most of Go protein contacts with flexible water. The later protein dangles in the membrane and can relax at a time scale much larger than what can be simulated with our computational resource. Nevertheless, these are very slow relaxation motion and weakly coupled with binding event of opioid. They are needed in a more comprehensive study of systematic biological signaling pathway, which is beyond the scope of this work. At our level, where we investigate the binding properties of μOR to potential ligands, the stability of μOR is the most important aspect.

3.2. Fluctuation of each residue in μOR

To additionally investigate the stability of μOR, the root mean square fluctuation (RMSF) of each residue are calculated. The result is shown in Figure 3. The average fluctuation value of backbone of each residue varies around 0.05-0.3 nm. As a member of GPCR family or so-called 7 transmembrane domain receptors (7TMR), μOR has 7 transmembrane helices (TM), three intracellular loops (ICLs), three extracellular loops (ECLs), an extracellular N-terminal and an intracellular C-terminal domain. The fluctuation of μOR in our model is consistent with the structure of μOR. 7 TMs show low fluctuation because they are restrained by the membrane. While other parts, located in water, fluctuate much larger than TMs. Notably, the residues Cys142 in the upper part of TM3 and Cys219 present in ECL2 considerably show lower fluctuation compared with neighbor residues. This is resulted from the disulfide bridge between these two residues. Some studies show that the mutations of μOR in these residues, disrupting disulfide bond, makes opioid ligands bind poorly to protein. Therefore, the stability effect of this disulfide linkage on ECL2 and upper part of TM3 has important role in ligand binding.

3.3. Secondary structure of μOR during the simulation

Secondary structure of μOR is stable during the simulation time (Figure 4). Focusing on the transmembrane regions, in several TMs including TM1, TM5, TM6, TM7, there are the structures of π-helix, 3_10-helix and turn appearing among α-helix structures. Generally, those
Figure 3: RMSF of protein backbone for \( \mu\)OR (green line). Residues CYS142 and CYS219 are marked as purple points. Bold lines along x-axis represent the transmembrane helices of \( \mu\)OR relative to the residue position.

Figure 4: Secondary structure as function of time for \( \mu\)OR as calculated by DSSP. The structures were analyzed every 200 ps. The secondary structure corresponding to each color is shown at the bottom.

kinds of structure are much more flexible than \( \alpha\)-helix. Indeed, \( \pi\)-helix usually appears in the middle of TM1, this leads to the fact that half of TM1 which is near extracellular side fluctuates more than the other part. This may be caused by the residue GLY84 and GLY87 in this TM, because glycine whose side chain contains only one hydrogen atom is highly flexible. Similarly, TM5 also has \( \pi\)-helix structure throughout the simulation time, the residues presenting \( \pi\)-helix are next to PRO246, it is worth to note that like glycine, proline which lacks backbone hydrogen
bond donor is also well-known as \( \alpha \)-helix breaker. In TM6, turn structure sometimes appears, while, in TM7, from 30 ns, turn structure maintain until the end of the simulation. Proline and glycine residues (PRO297 - TM6, GLY327, PRO 335 - TM7) are believed to contribute to the formation of unstable structure. In addition, these two proline residues belong to the "H-bonder/Proline" motif, where proline kink can be regulated by a residue with hydrogen bonding potential. In \( \mu \)OR, the TP (THR296-PRO297) and NP (ASN334-PRO335) motif will enhance the proline kink [17]. Actually, transmembrane proline and glycine were demonstrated to play an important role in the function of transmembrane protein [18, 19]. Our molecular simulation result provides strong support to these experimental hypotheses.

4. Conclusion
In this paper, using molecular dynamics simulation, we studied the relationship between the structure and the dynamic properties of \( \mu \)OR. Our results showed that the disulfide bond between CYS142-CYS219 decreases the fluctuation of TM2 and ECL3, this effect supports the experimental studies which showed that mutations disrupting disulfide bond of \( \mu \)OR reduce opioids binding. In addition, by analyzing the secondary structure of \( \mu \)OR during the simulation and the presence of proline and glycine residues, we clarified the essential role of transmembrane proline and glycine in increasing the flexibility of transmembrane helices which will diversify the signals of \( \mu \)OR formed by interaction with different ligands. For further research, we will study the impacts of different opioids including biased and unbiased ligands on the dynamics and conformations of GPCRs.

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