Biochimie 123 (2016) 52–64

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Investigating the structural impact of S311C mutation in DRD2 receptor by molecular dynamics & docking studies

Avijit Podder, Deeksha Pandey, N. Latha*

Bioinformatics Infrastructure Facility, Sri Venkateswara College (University of Delhi), Benito Juarez Road, Dhaula Kuan, New Delhi, 110 021, India

ARTICLE INFO

Article history:
Received 19 August 2015
Accepted 16 January 2016
Available online xxx

Keywords:
Dopamine receptor D2 (DRD2)
S311C mutation
Molecular docking
Molecular dynamics (MD) simulations
Residue interactions network (RIN)

ABSTRACT

Dopamine receptors (DR) are neuronal cell surface proteins that mediate the action of neurotransmitter dopamine in brain. Dopamine receptor D2 (DRD2) that belongs to G-protein coupled receptors (GPCR) family is a major therapeutic target for of various neurological and psychiatric disorders in human. The third inter cellular loop (ICL3) in DRD2 is essential for coupling G proteins and several signaling scaffold proteins. A mutation in ICL3 can interfere with this binding interface, thereby altering the DRD2 signaling. In this study we have examined the deleterious effect of serine to cysteine mutation at position 311 (S311C) in the ICL3 region that is implicated in diseases like schizophrenia and alcoholism. An *in silico* structure modeling approach was employed to determine the wild type (WT) and mutant S311C structures of DRD2, scaffold proteins – $G_{\alpha i/o}$ and NEB2. Protein-ligand docking protocol was exercised to predict the interactions of natural agonist dopamine with both the WT and mutant structures of DRD2. Besides, atomistic molecular dynamics (MD) simulations were performed to provide insights into essential dynamics of the systems-unbound and dopamine bound DRD2 (WT and mutant) and three independent simulations for $G_{\alpha i}$, $G_{\alpha o}$ and NEB2 systems. To provide information on intra-molecular arrangement of the structures, a comprehensive residue interactions network of both dopamine bound WT and mutant DRD2 protein were studied. We also employed a protein–protein docking strategy to find the interactions of scaffold proteins – $G_{\alpha i/o}$ and NEB2 with both dopamine bound WT and mutant structures of DRD2. We observed a marginal effect of the mutation in dopamine binding mechanism on the trajectories analyzed. However, we noticed a significant structural alteration of the mutant receptor which affects $G_{\alpha i/o}$ and NEB2 binding that can be causal for malfunctioning in cAMP-dependent signaling and Ca^{+} homeostasis in the brain dopaminergic system leading to neuropsychiatric disorders.

1. Introduction

The dopaminergic neurotransmission is mediated by dopamine receptors (DR) in human brain [1]. Dopamine receptors belong to the rhodopsin like family of G-protein coupled receptors (GPCR) which consists of the characteristic extracellular amino terminus, an intracellular carboxyl terminal region and seven transmembrane (TM) α-helices spanning the membrane. The TM domains are connected by three intracellular loops (ICL) and three extracellular loops (ECL) [2]. Broadly, dopamine receptors are of five types (DRD1-5) classified on the basis of structural, biochemical and pharmacological properties. They are sub-classified into D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3 and DRD4) family based on their different ability to activate G proteins [3,4]. Dopamine receptor D2 (DRD2) of D2-like family is believed to be crucial for learning and memory in brain [5]. DRD2 is widely expressed in human brain and is localized in both pre and post synaptic region [6]. DRD2 has two distinct molecular variants – D2-long and D2-short, produced by alternative splicing that eliminates a stretch of 29 amino acid residues in the ICL3 of DRD2-short. The DRD2-short is majorly a presynaptic receptor, and less relevant in $G_{\alpha i/o}$ protein coupling whereas DRD2-long is a postsynaptic receptor [7–9]. These variants are pharmacologically similar except for small differences in their affinity for specific G-proteins [10]. DRD2 mediated second-messenger signal transduction cascade is well established where the natural agonist dopamine binds to the TM region of DRD2 protein. This binding activates specific guanine

* Corresponding author.
E-mail address: lata@bic-svc.ac.in (N. Latha).

http://dx.doi.org/10.1016/j.biochi.2016.01.011
0300-9084/

binding proteins ($G_{\alpha i}$ and $G_{\alpha o}$) at ICL region [11,12], thereby inhibiting cAMP production [13,14]. Apart from $G_{\alpha i/o}$ protein mediated signaling, DRD2 is also involved in several other cAMP-independent signaling pathways [15]. One such process involves the association of neurabin (NEB2) with the third cytoplasmic loop (ICL3) of DRD2 that regulates the Ca^{+} signaling [16,17]. NEB2 serves as an important scaffold protein in DRD2 mediated post-synaptic signaling.

Polymorphisms in dopamine receptor genes have the potential to affect disease susceptibility and response to therapeutic agents [18]. Mutation studies have reported that the polymorphic variants present in ICL3 have differential ability to bind and activate specific G-protein [19]. One such deleterious mutation at position 311 (S311C) in ICL3 region of DRD2 is to have decreased affinity for dopamine and G-protein [20]. Recent genomes wide association studies (GWAS) in family based [21,22] as well as in independent case–control cohorts [23–36] have reported this mutation to be associated with higher risk of schizophrenia and alcoholism though few published evidences also negate such association [37–39]. Evidences from available GWAS have been summarized in Table S1. S311C mutation is also being widely focused for DRD2 specific drug design approaches due to its growing clinical relevance for the treatment of psychiatric disorders [40–42].

The bottleneck in understanding the structure–function relationship of any GPCR is the difficulty to determine the conformation of the GPCR *in vivo* [16]. The membrane bound structure of GPCRs makes it challenging to procure sufficient quantities for protein crystallization. To compensate that, biophysical approaches such as molecular dynamics (MD) simulations provide an alternative tool to define the time dependent conformational behavior of GPCRs structure [43–46]. The lack of experimental three dimensional (3-D) structures (X-ray crystallography or Nuclear Magnetic Resonance (NMR)) of human DRD2 protein enthused us to predict its structure. In this context, it is important to mention that we have given preference on DRD2-long structure only in our study because of its prevalence for postsynaptic signal transduction. We have refined the modeled structure using MD simulations to examine the conformational preference of the disease causing S311C mutation. Molecular docking studies with endogenous ligand dopamine and signaling proteins $G_{\alpha i/o}$ and NEB2 provided valuable insights to the functional basis of the mutation in DRD2 mediated post synaptic signaling cascade. To the best of our knowledge, this study is a unique effort to evaluate the possible role of S311C mutation for DRD2 structural alteration that can be a reason for the functional association of the mutation in certain psychiatric disorders.

2. Methodology

2.1. Structure prediction and validation

The sequence of human DRD2 protein (443 amino acids) was retrieved from UniProt database (http://www.uniprot.org/uniprot/P14416) and the 3-D structure was modeled using the I-TASSER (Iterative Threading ASSEmbly Refinement) web interface [47]. I-TASSER use a combinations of various approaches such as threading, fragment assembly, *ab initio* loop modeling and structural refinement to predict the structures. The crystal structure of human DRD3 protein (PDB ID: 3PBL) that has a sequence identity of 53% was taken as a primary template to model the structure of DRD2. Based on the confidence score (C-score), the best model was selected among all five predicted models posed by I-TASSER. Additionally, structural models of human GNAI1 ($G_{\alpha i}$), GNAO1 ($G_{\alpha o}$) and NEB2 (PPP1R9B) proteins were also determined by employing the I-TASSER protein modeling pipeline. The predicted C-score for all the final structures are listed in Table S2. To assess the reliability of all the predicted models, Structural Analysis and Verification Server (SAVES) (http://nihserver.mbi.ucla.edu/SAVES) was exercised. We calculated the backbone conformation and overall stereochemical quality of the modeled structures by analyzing the phi (Φ) and psi (ψ) torsion angles through PROCHECK program [48] to cross-check the Ramachandran plot statistics. The non-bonded atomic interactions were examined using ERRAT program [49]. Additionally, the modeled 3-D structure of human DRD2 protein was also validated by prefiguring the binding sites and relative binding affinities for endogenous ligand dopamine with the documented experimental observations.

We used SIFT [50], Polyphen-2 [51], SNPeffect [52] and I-Mutant Suite [53] tools to compute the disease causing effect of the non-synonymous mutation S311C in DRD2 protein. To build the mutant structure, a point mutation in Wild Type (WT) DRD2 structure at position 311 from serine to cysteine was incorporated using SPDB Viewer package [54]. Prior to docking, final energy minimization and refinement of the structures were done followed by steepest descent (100 steps) and conjugate gradient (500 steps) method from SPDB viewer software platform.

2.2. Molecular docking protocol

2.2.1. Protein-ligand docking

Both the WT and mutant modeled structures of DRD2 protein were docked with the natural agonist dopamine through a flexible docking protocol using Glide (Schrödinger) [55]. The chemical structure of doapmine was drawn in 2D sketcher (Maestro version 9.3) [56]. Ligand preparation was done using Ligprep (Schrödinger) set at default parameters (Ionizers, generating tautomers, generating possible conformers at pH 7 with OPLS_2005.1 force filed) [57]. The geometries of the generated structures were optimized to ensure the correct protonated state of dopamine. Prior to docking, experimentally reported ligand binding sites were used to define active site in DRD2 structure. Dopamine was docked into the active sites of modeled DRD2 structures using the “extra-precision” (XP) mode of the Glide cross docking program [55]. Multiple conformers of dopamine were docked eventually with both the WT and mutant structures of DRD2. A binding free energy (ΔG_{bind}) for each docked pose was further estimated using Prime MM-GBSA method (Prime, version 3.1) [58]. The ΔG_{bind} was estimated as

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} + \Delta G_{SA}$$

where ΔE_{MM} is the difference in energy between the dopamine bound complex structure of DRD2 and the sum of the energies of the dopamine and unliganded DRD2. ΔG_{solv} is the difference in the GBSA solvation energy of the receptor–ligand complex and the sum of the solvation energies for the dopamine and unliganded DRD2 and ΔG_{SA} is the difference in surface area energies for the complex and the sum of the surface area energies for the unliganded DRD2 and dopamine [59]. Complex with the lowest glide score was refined by ΔG_{bind} score.

2.2.2. Protein–protein docking

To compare the interactions of WT and mutant DRD2 with $G_{\alpha i/o}$ and NEB2 proteins, we employed a rigid body protein–protein docking protocol of ClusPro web server (version 2.0) [60]. ClusPro is designated as top-ranked web server according to latest round of CAPRI (Critical Assessment of Predicted Interactions) experiment for protein–protein docking [61]. We calculated the interface energy (ΔiG) using PDBePISA tool [62]. A global energy (ΔG) for each docked pose was refined with FiberDock web server [63]. Module DIMPLOT of LIGPLOT facilitated the visualization of the interacting residues in the Protein–Protein interface [64].