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Understanding the inhibitory mechanism of BIT225 drug against p7 viroporin using computational study

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HIGHLIGHTS

- Molecular dynamics simulations were done to investigate the inhibition mechanism of BIT225.
- The hydrophobic interaction played a crucial role in BIT225 binding to p7 channel.
- The structural rearrangement of p7 channel plays a key role in channel activity.
- BIT225 binding to p7 channel can restrict the structural rearrangements of the channel.

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ABSTRACT

P7 is the only viral channel encoded by the Hepatitis C Virus (HCV) genome. It is a small, highly hydrophobic protein containing 63 amino acids. Structural studies have shown that p7 has two transmembrane (TM) α helices linked by a short dibasic cytoplasmic loop. P7, mostly placed in the endoplasmic reticulum (ER), is a membrane-associated protein. The results obtained from different studies revealed that p7 is a polytopic membrane protein that could oligomerize in membrane bilayer to create ion channels with cation selectivity. In addition, p7 is highly conserved and plays an important role in the assembly and release of mature viral particles. Thus, it can be considered as a potential target for anti-HCV drugs. It has been found that several compounds (amantadine, rimantadine, hexamethylene amiloride (HMA) and long-alkyl-chain iminosugar (IS) derivatives) inhibit p7 channel ability. Another new inhibitor identified as BIT225, a derivative of amiloride, also inhibits the viroporin function of HIV-1 Vpu and HCV p7. In the present study, molecular dynamics simulations were applied to get insights into molecular details of a BIT225 binding site. In addition, the g_mmpbsa approach was employed to calculate the binding free energy and free energy decomposition per residue. MD simulation results in the p7–BIT225 complex revealed that drug binding to hydrophobic pocket can allosterically inhibit ion conduction via the funnel tip by restricting significant intrinsic channel breath at the tip of the funnel. Based on the molecular dynamics simulation (MD) analysis and the energy profiles, the hydrophobic interactions were the main driving force for BIT225 binding.

1. Introduction

The hepatitis C virus (HCV) causes both chronic and acute liver disease [1]. In most individuals infected with hepatitis C virus, it becomes a chronic infection. Chronic hepatitis C is a serious liver infection that can cause long-term health problems (cirrhosis, hepatic fibrosis), even death [2,3]. In most cases, the infected person may not be aware of his disease since he is not patient clinically. Nowadays, there are about 170 million people infected with HCV around the world and 3–4 million new infections occur each year [3].

The p7 of HCV is a small highly hydrophobic protein along with 63 residues. Studies on the subcellular position of HCV proteins show that p7 is located in the membrane of endoplasmic reticulum (ER) [4–7]. Thus p7 is membrane-associated protein [7]. HCV p7 protein has two transmembrane domains linked by a small basic cytoplasmic loop. P7 integral membrane polypeptide is a double membrane-spanning topology, the N and C terminal tails are oriented toward the ER lumen [5,6]. HCV p7 polytopic membrane protein creates cation ion channels in phospholipid bilayers [6,7]. The final structure of p7 channel is similar to flower shape where hydrophilic residues create the luminal side of the pore and hydrophobic residues make contact with lipids [4–7]. Recent studies showed that the p7 is very important in the

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facilitation of cation permeability across membranes as well as the assembly and release of viral mature particles [6–8]. Thus, p7 is a potential target for anti-HCV drugs. Several compounds such as amantadine and rimantadine [9], hexamethylene amiloride (HMA) [10], and the long-alkyl-chain iminosugar derivatives [11] have been clarified to inhibit channel activity of the p7 viroporin.

Another novel inhibitor molecule identified as BIT225, a derivative of amiloride, also inhibits the viroporin function of HIV-1 Vpu and HCV p7. BIT225 (*N*-[5-(1-methyl-1H-pyrazol-4-yl)-napthalene-2-carbonyl]-guanidine) was developed by Biotron (Murdoch, WA, Australia) [12]. Additionally, BIT225 shows a synergistic effect in HCV infections with interferon a2b in vitro [12]. Recent reports provided evidence that BIT225, among viroporin inhibitors, has the most inhibitory effect on the p7 channel [12,13]. Moreover, BIT225 shows the lowest binding energy compared to other p7 channel inhibitors such as NN-DNJ, amantadine and rimantadine in docking studies [14]. Previous NMR studies indicated that the adamantane (rimantadine and amantadine) inhibitors bind to six equal hydrophobic pockets. Each pocket contains F20, V25 and V26 from H2, and L52, V53 and L56 from H3 [4]. Recent studies showed that binding adamantine inhibitors to these hydrophobic pockets could operate as “molecular wedge” that decreases conformational dynamics required for cation flow via the N-terminal restriction of p7 channel [15,16]. Another study conducted on the p7 channel showed that power inhibition of HMA is stronger than rimantadine at similar inhibitor concentration [17]. Previous NMR studies suggested that HMA, amantadine and NN-DNJ induced more chemical shift perturbations in the loop region and terminal regions, respectively [18]. NMR based drug-binding experiments suggest that the presence of rimantadine, amantatadine and HMA can allosterically inhibit ion conduction via the funnel tip by restricting significant intrinsic channel breath at the tip of the funnel [15–18]. Moreover, these studies suggested that NN-DNJ did not bind to p7 channel, but NN-DNJ potentially disturbing oligomerization by docked to protomer interface of the p7 monomers [19].

This study aimed to investigate the mechanism by which BIT225 inhibits the activity of p7 channel of hepatitis C virus through methods like docking, comparative molecular dynamics simulations, and binding free energy calculation. Computational approaches such as molecular dynamics simulations, molecular docking, and binding energy calculation have been recently shown to be successful and beneficial in understanding the molecular details of drugs' mechanism and their resistance to diverse antiviral agents [20,21]. These methods allow us to calculate energetic data and structural features of drug-protein binding.

The p7 viroporin is inserted into a membrane of POPC (Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine). Finally, molecular dynamics simulations of channel/lipid system and channel-drug/lipid system were conducted in a buffer with an ionic strength of 1 M under external electric field.

2. Materials and methods

2.1. Molecular docking details

The 3D structure of the p7 channel was obtained from Protein Data Bank (2m6x) [4]. The p7 ion channel complex with the BIT225 was built by the Auto dock vina [22]. P7 channel and BIT225 parameter files were created by AutodockTools-1.4.5 [23]. A grid of 90, 87 and 100 in x, y and z directions, respectively, surrounding BIT225 binding site was set up with a grid spacing of 0.375. Finally, optimized docked conformations were selected on the basis of binding energy and applied as initial structure for molecular dynamics simulations.

2.2. Molecular dynamics details

All the MD simulations were performed by the gromacs-4.6.4 package using the GROMOS96 force field. The BIT225 topology file for Gromacs was provided using the program Topolbuild, developed by Bruce D. Ray. POPC membrane patches were generated from 16:1 to 18:1 Diester PC, 1-Palmitoyl-2-Oleoyl-*sn*-Glyucero-3-Phosphocholine molecules on the basis of parameters of Chandrasekhar et al. [24]. The solvent molecules were characterized by simple point charge (SPC) model and the protein/lipids were described by Gromos96 ffG45a3 force field. At first, the POPC membrane model was equilibrated for 50 ns, then the complex of channel-drug and the p7 channel was separately inserted into the membrane. All systems were solvated with explicit solvents (SPC). Chloride (Cl^-) counter-ions were added to neutralize each system. Next, the energy minimization (EM) was performed by the steepest descent algorithm [25]. Then, each system was gradually heated up from 0 to 310 K over 1 ns in the NVT ensemble and equilibration to adjust the solvent density under 1 atm pressure over 1 ns in the NPT ensemble simulation by restraining all atoms of the structures. Then, main runs were performed without any restraint on these seven systems in the NPT ensemble at a temperature of 310 K and a pressure of 1 atm to coordinate trajectory was recorded every 0.01 ns during the equilibration and main runs. During the simulations, periodic boundary conditions (PBC) were implemented. Long-range Coulombic interactions were assessed using the PME (particle mesh Ewald) [26] summations. In the equilibration and next production runs, the internal degrees of freedom of the solvent molecules were restrained by the SHAKE algorithm [27] and all bond lengths in the macromolecules were restrained via the LINCS algorithm [28]. The channel/membrane system was then extracted from final frames of the main run, and solvent molecules were substituted with ions (Ca^{2+}, Cl^-) to reach the 1 M ionic strength. After this step, the whole system was equilibrated to 10 ns and presented to the main run. Moreover, we used an external electric field perpendicular to the bilayer xy plane to the whole system in the simulation box [29]. The channel-BIT225 complex structure obtained from docking phase was also applied as an initial structure for performing MD simulations by a similar procedure as declared in the channel/membrane system.

2.3. MD trajectory analysis

The simulation trajectories were analyzed by g_rmsf, g_rms, and do_dssp of GROMACS utilities [30]. Plots and images were generated using xmgrace, PyMol, [31] and LIGPLOT software [32], respectively.

2.4. Binding profile energy and residue decomposition studies

Binding energy was calculated by MMPBSA approach utilizing the g_mmpbsa tool [33]. In g_mmpbsa, the binding free energy of a protein to a ligand is defined as $\Delta G_{binding} = G_{complex} - (G_{protein} + G_{ligand})$, where the terms on the right side of the equation represent the total free energies of the protein-ligand complex and the isolated protein and ligand in the solvent, respectively. Moreover, the free energy for each individual entity (i.e., the complex, the protein, or the ligand) is given by $G = < E_{MM} > - TS + < G_{sol} >$. The terms $< E_{MM} >$ and $< G_{sol} >$ represent the average potential energy in vacuum calculated with molecular mechanics force field and solvation free energy, respectively [33].

$$E_{MM} = E_{bonded} + E_{nonbonded}$$

$$E_{bonded} = E_{bond} + E_{angle} + E_{torsion}$$

$$G_{sol} = G_{sol\text{-}ele} + G_{sol\text{-}np}$$

The bonded energy (E_{bonded}) includes angles, bond and torsion energies. The Enonbonded are nonbonded contacts, containing van der Waals (EvdW) and electrostatic (Eelec) interactions and are modeled by a Lennard-Jones (LJ) and Coulomb potential function, respectively [33]. Also, contribution energy of residues could be calculated using