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Exploring the selectivity of auto-inducer complex with LuxR using molecular docking, mutational studies and molecular dynamics simulations

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ABSTRACT

Bacteria communicate with one another using extracellular signaling molecules called auto-inducers (AHLs), a process termed as quorum sensing. The quorum sensing process allows bacteria to regulate various physiological activities. In this regard, quorum sensing master regulator LuxR from *Vibrio harveyi* represents an attractive therapeutic target for the development of novel anti-quorum sensing agents. Eventhough the binding of AHL complex with LuxR is evidenced in earlier reports, but their mode of binding is not clearly determined. Therefore, in the present work, molecular docking, *in silico* mutational studies, molecular dynamics simulations and free energy calculations were performed to understand the selectivity of AHL into the binding site of LuxR. The results revealed that Asn133 and Gln137 residues play a crucial role in recognizing AHL more effectively into the binding site of LuxR with good binding free energy. In addition to that, the carbonyl group presents in the lactone ring and amide group of AHL plays a vital role in the formation of hydrogen bond interactions with the protein. Further, structure based virtual screening was performed using ChemBridge database to screen potent lead molecules against LuxR. 4-benzyl-2-pyrrolidinone and *N*-[2(1-cyclohexen-1-yl) enthyl]-N'(2-ethoxyphenyl) were selected based on dock score, binding affinity and mode of interactions with the receptor. Furthermore, binding free energy, density functional theory and ADME prediction were performed to rank the lead molecules. Thus, the identified lead molecules can be used for the development of anti-quorum sensing drugs.

1. Introduction

The term quorum sensing (QS) refers to the process of intercellular bacterial cell-cell communication that influences the expression profile of diverse genes in a cell density dependent manner [1–3]. Both Gram negative and Gram positive bacteria use QS communication circuits to regulate the expression of various virulence factors, bioluminescence, horizontal transfer of DNA, sporulation, pigment production, antibiotic production and biofilm formation [4–6]. In this regard, QS system represents an attractive target for the development of novel anti-microbial agents. Many groups of researchers have been engaged in identifying potent inhibitors targeting LuxR of *Vibrio harveyi* [7,8]. The QS system not only regulates the virulence related genes but also regulate genes that are involved in the basic metabolic process. A significant portion of bacterial genome (4–10%) and bacterial proteome (20%) are influenced by QS [9]. The phenomenon of QS was first recognized in marine bacterium *Vibrio fischeri* [10].

In most Gram negative bacteria, LuxI-family of protein synthesizes a specific *N*-acyl-L-homoserine lactone signaling molecule (AHL) called auto-inducer [11]. Increase in bacterial population density concordantly increases the concentration of AHLs in the environment [12]. Depending on the bacterial species, the acyl side chain of AHL can vary from 4 to 18 carbons in length. The acyl chain contains oxo, hydroxyl or no substitution on the third carbon atom [13]. Once the maximum concentration of AHL is reached, it binds with LuxR, and then activates the gene expression levels for the formation of biofilm, bioluminescence, motility etc. Numerous reports have proposed that LuxR family of proteins has two domains, the *N*-terminal domain binds the AHL, while the *C*-terminal domain

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involves in the DNA binding [14,15]. To understand the interactions between AHL and LuxR, computational techniques were used to gather information on the origin of selectivity. An interference with AHL mediated gene expression represents an attractive path for the control of unwanted microbial activity without interfering with the growth of the bacterium. Many researchers are focusing to identify analogues for AHL and that could function as auto-inducer antagonists.

In this present study, molecular docking combined with *in silico* mutational analysis, binding free energy and molecular dynamics (MD) simulations were performed to investigate the selectivity of AHL in complex with LuxR. In addition, we have identified lead molecules targeting LuxR using structure based virtual screening approach. Furthermore, the identified lead molecules were ranked based on DFT (density functional theory), ADME (absorption, distribution, metabolism and excretion) and free energy calculations. Hence, this practice has importance in the development of novel anti-quorum sensing drugs.

2. Materials and methods

All computational studies were carried out in a single machine running on Intel Core TM 2 Duo processor with 2 GB RAM and 160 GB hard disk with Red Hat Linux Enterprise version 5.0 as the operating system.

2.1. Multiple sequence alignment and phylogenetic tree analysis

The amino acid sequence of LuxR proteins from *Vibrio* species were downloaded from GenBank database. Multiple sequence similarity search was performed using ClustalW program implemented in Mega 5.1 [16]. The aligned sequences were used as an input for the construction of phylogenetic tree using Maximum likelihood method. Jone-Taylor-Thornton amino acid substitution matrix was used to estimate the evolutionary distances between the sequences. Robustness of the tree topology was measured by testing the phylogeny, bootstrap replication test with 500 steps was carried out with default parameters. Three dimensional structures were predicted using PS2 server [17] to understand the secondary structural similarities among the sequences. Finally, the LuxR sequence (accession number: Q8GBU2) from *Vibrio harveyi* was selected and its physicochemical parameters were estimated using ExPASy proteomics tool [18].

2.2. Protein and ligand preparation

The LuxR protein information's were taken from our published data [7]. The structure of AHL and lead molecules obtained from ChemBridge database were drawn using Marvin sketch. Energy minimization was performed by applying optimized potential for liquid simulation (OPLS)-2005 force field with default setting followed by preparation using LigPrep module of Schrödinger (LigPrep, 2013) [19]. Missing hydrogen atoms were added and optimized, refinement of bond orders, formal charges and orientation of various functional groups were fixed and then the ligands are neutralized. Maximum of 32 stereoisomers were generated per ligand and the most relevant ionization and tautomeric states were generated at pH 7.0 ± 2.0 with Epik module. For each ligand, low energy ring conformations were generated and optimized ligands were further used for docking studies.

2.3. Molecular docking

Two types of docking protocols such as Rigid Receptor Docking (RRD) [20] and Induced Fit Docking (IFD) [21] were used to dock the AHL onto the refined LuxR model. Binding site information for LuxR was retrieved from our previous published data [7]. In RRD, the internal geometry of the protein is fixed while the ligand (AHL act as a ligand) is flexible to explore the arbitrary number of torsional degrees of freedom in addition to the six degrees of freedom spanned by the translation and rotational parameters. The docking was carried out using Glide (grid-based ligand docking with energetics) extra precision (XP) mode at default parameters. Initially, the process involves in the fitting of ligand into the defined active site of the protein and then examines the complementarily of ligand-protein interactions using grid-based method patterned after the empirical ChemScore function. The best ligand was refined via Monte-Carlo sampling and further minimization was used to obtain an accurate docked pose. Five poses were generated for the ligand and the resulting docked conformations were analyzed with Glide pose viewer tool. In order to consider the flexibility of both protein and ligand, the IFD protocol was adopted. Initially, the docking of ligand with LuxR was performed using soften potential (van der Waals radii scaling) in the Glide program. A van der Waals radii of LuxR protein was scaled to 0.5 and a maximum of 20 poses per ligand were generated, the poses retained must have a coulomb-van der Waals score less than 100 and hydrogen bond score less than −0.05. Each ligand-protein complex was then refined and minimized with Prime module. The receptor in each pose now reflects an induced fit to the ligand structure. Finally, Glide re-docking of each protein-ligand complex was performed with the refined low energy receptor structure. The binding affinity of each complex was ranked according to the glide score. The best docked conformations were chosen based on scoring functions and maximum number of hydrogen bond interactions that mediate between protein and ligand.

2.4. Molecular dynamics (MD) simulations

MD simulations were carried out for LuxR-AHL (protein-ligand) complex as well as three mutant forms of LuxR (Asn133Ala, Gln137Ala, Asn133Ala and Gln137Ala) bound AHL complexes to understand the protein-ligand interactions and its stability using GROMACS v4.5.5 package (Groningen Machine for Chemical Simulation) with GROMOS96 43A1 force field [22]. The ligand topology file was generated using PRODRG web server. The protein-ligand complexes were solvated in cubic box with simple point charge (SPC)-216 water environment. Five appropriate Na^+ counter ions were added to neutralize the systems. In order to remove the unwanted van der Waals contacts, the solvated system was energy minimized using hybrid method of steepest descent minimization followed by limited memory broyden fletcher goldfarb shanno algorithm. After energy minimization, the system was simulated with isothermal-isochornic (NVT) ensemble by applying constant temperature of 300 K using Berendesen thermostate with the time set of 0.1 ps. Then in the isothermal-isobaric (NPT) ensemble, the pressure of 1 atm was maintained using Martyna-Tobias Klein method with the time set of 0.5 ps. Covalent bonds involving hydrogen atoms were constrained using LINCS (Linear constraint solver) algorithm [23] and Particle Mesh Ewald method for electrostatics [24]. SETTLE algorithm was used to constrain the geometry of all covalent bonds containing water molecules. After equilibration, the minimized system was subjected to 10 ns long MD simulations. All trajectories were recorded for every 1.2 ps. The *C-α* Root Mean Square Fluctuation (RMSD), Root Mean Square Fluctuation (RMSF) and hydrogen bond interactions in each trajectories were analyzed with respect to time scale. Plots were graphically analyzed using Origin Pro.