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Structural dynamics of LasR protein in the regulation of hcnABC operon

Dynamics of LasR protein

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Abstract: ***Pseudomonas aeruginosa* (*P. aureginosa*) is a human pathogen with opportunistic nature. It mainly attacks immune suppressed people. It produces hydrogen cyanide (HCN) as a potential virulence factor. From different studies it has been found that HCN poisoning can cause neuronal damage that can lead to many neurological disorders. In *P. aureginosa*, HCN is synthesized by HCN synthase enzyme, encoded by hcnABC operon. LasR is a vital transcriptional regular for *hcnABC* gene transcription. The LasR protein is activated by an autoinducer ligand named N-3-oxo-dodecanoyl-L-Homoserine lactone (OdDHL). The activated LasR monomer further dimerizes and binds to its cognate promoter DNA, named lux α, and facilitates the transcription of *hcnABC* genes. In this work we have tried to explore the involvement of the OdDHL ligand in the activity of transcriptional regulator dynamically, to map the activity LasR transcriptional regulation.**

***Index Terms*—Protein Modelling, Docking, MD Simulation, Autoinducer, Transcription.**

INTRODUCTION

Pseudomonas aeruginosa is a human pathogen with opportunistic nature. It mostly attacks humans with suppressed immune system, like people suffering from cancer, AIDS, cystic fibrosis [1]. They attack their target with some virulence factor produced by them. Hydrogen cyanide (HCN) is one potential virulence factors produced by *P. aeruginosa* [2]. From study of cyanide poisoning in human and animal, it has been found that HCN can cause strong neuronal damage, like neuronal necrosis that can lead to many neuronal disorders or something lethal. In a study on *C. elegans,* it has been found that *P. aeruginosa* can paralyze and kill *C. elegans* [3].

P. aeruginosa produces HCN with the help of a membrane bound enzyme named HCN synthase. Three genes, *hcnA, hcnB, hcnC*, forming hcnABC operon, encode this enzyme [4]. Three transcriptional factors mediate the transcription of these *hcnABC* genes, viz., LasR, ANR, RhlR. These three transcription regulators function in optimum level when in clustered form. Here we will focus on the first transcriptional regulator, LasR [5].

LasR is a transcriptional regulator protein belonging to the LuxR transcriptional regulator family. It regulates the target gene by recognizing and binding to its cognate promoter sequence, named lux α [6], [7]. An activation process triggers this protein to function. This activation is done by the binding of an autoinducer ligand named, N-3-oxo-dodecanoyl-L-Homoserine lactone (OdDHL) to the LasR protein. This activation triggers LasR protein to be dimerized. Dimerization of LasR is very much necessary for the binding with the promoter DNA. Hence the ligand binding is very much necessary for activity of LasR, for dimerization as well as DNA binding [8]. This protein has two distinct domains, the N-terminal ligand binding domain, and the C-terminal domain, which possess a helix turn helix(HTH) motif, which helps it to recognize and bind the promoter DNA sequence [9].

In this work we have tried to explore the dynamic nature of LasR protein during dimerization in presence of the autoinducer ligand (OdDHL), and what happend to the dimerization process if the ligand is absent. This study can help to map the activity of LasR in hcnABC genes regulation which is responsible for HCN synthesis. This map can further help to disrupt the activity of LasR transcriptional regulator from proper functioning.

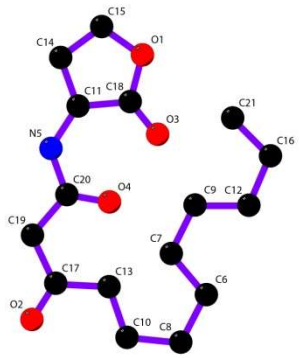
MATERIALS AND METHODS

Protein Modelling

In order to study the dynamics of the LasR dimer, we need to build the homology model of LasR, as the crystal structure of LasR(PDB ID - 3IX3) in Brookhaven Protein Data Bank (PDB) [10] was not complete. So, to build this model we took the amino acid sequence from NCBI (Seq. ID - NP_250121.1). The LasR protein has 239 amino acid residues. To find a suitable template for model building, we performed protein BLAST [11]. From the BLAST report, the best template was found to be 3SZT, Chain A with 30% sequence identity, 97% query coverage, and E-value $1x10^{-25}$. The homology modeling of this LasR protein was done by HHPred web server [12]. The stereo-chemical fitness of this modeled structure was validated using PROCHECK [13], Vreify3D [14] (score 99.58%) and ProSA [15] (Z-score -6.63). No amino acids were in disallowed regions in Ramachandran plot analysis.

Ligand Protein Docking

The structure of ligand (OdDHL) (Figure 1) was obtained from PubChem web server (PubChem compound ID = 3246941) [16]. The ligand(OdDHL) was docked with the LasR monomer by cavity detection method with the Ligandfit tool in Discovery studio (DS).



N-(3-Oxododecanoyl)homoserine lactone

Fig. 1. N-3-oxo-dodecanoyl-L-Homoserine lactone, the ligand molecule.

Dimerization

The dimerization of the LasR monomer was done in Z-dock web server [17]. This web server performs rigid body docking. The dimerization of the lasR protein was done in two conditions. First we dimerized the LasR protein using the LasR monomer bound to its ligand (OdDHL). And secondly we dimerized the LasR protein using the LasR monomer having no bound ligands (OdDHL) within it. Now we had two LasR dimers, one bound to its ligand, and the another dimer has no ligand bound.

Molecular Dynamics Simulation of LasR dimer

We performed molecular dynamic (MD) simulation for 50 nano seconds. The first MD simulation was done with the LasR dimer having the ligand bound, and the second MD simulation was done with the lasR dimer without ligand. This MD simulation was done using Gromacs 5.0.1 tool [18].

Docking of LasR Dimer with Promoter DNA

To build the model of the promoter DNA (lux α) of LasR transcription factor, we took the following sequence of lux α from literature [5].

5' ACCTACCAGAATTGGCAGGG 3'

The model was built in DS. The LasR dimer was docked with the promoter DNA using PatchDock web server [19].

RESULT AND DISCUSSION

We analyzed the MD simulation data in Gromacs 5.0.1. The following analyses were performed on the MD data: Root mean square deviation (RMSD), Root Mean Square Fluctuation (RMSF), the potential energy of the dimer throughout the simulation in presence and absence of the ligand (OdDHL), and comparative structural analysis of these two LasR dimers in presencet and absence of ligand.

RMSD

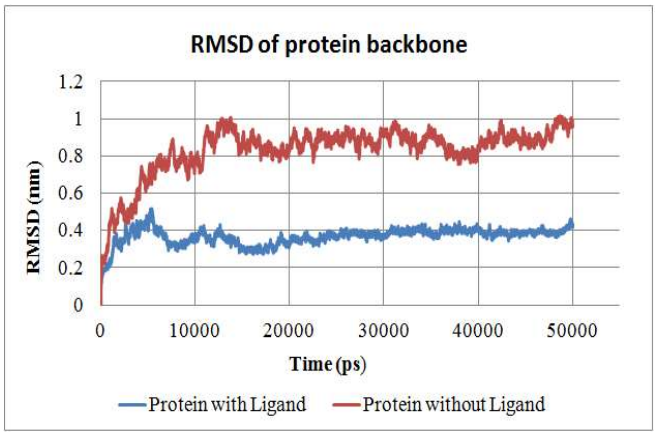


Fig. 2. Root mean square deviation of the protein backbone throughout simulation.

We calculated the RMSD of the protein backbone of these two proteins (one with ligand and another without ligand) and plotted them in a graph (Figure 2). We found that the initial RMSD value of these two dimer proteins were 0.17 Å. As we performed rigid body docking the initial RMSD was very low. Though the simulations of these two proteins were performed in identical conditions, from the RMSD values as appeared in the graph it was clear that the structural conformations were gradually deviating throughout the simulation time. In these two simulation systems the only difference was the presence and the absence of the ligand (OdDHL) in the protein dimer.

RMSF

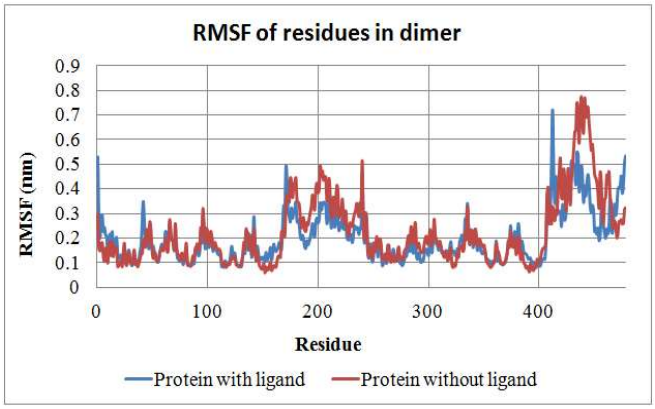


Fig. 3. Root mean square fluctuation of the protein residues throughout simulation.

We analyzed the RMSF data of the protein residues of the LasR protein in presence and absence of ligands throughout the simulation time. In each dimer there are 478 (2 x 239) residues. This RMSF data showed the fluctuation level of each residues during the simulation time. An unstable or a relatively unstable system would always show a higher fluctuation level to reach the stability. The RMSF data represented in the graph (Figure 3) showed that the amino acids present in the protein without ligand, had a comparatively higher fluctuation in different regions in respect to the protein bound to its ligand.

System Potential Energy

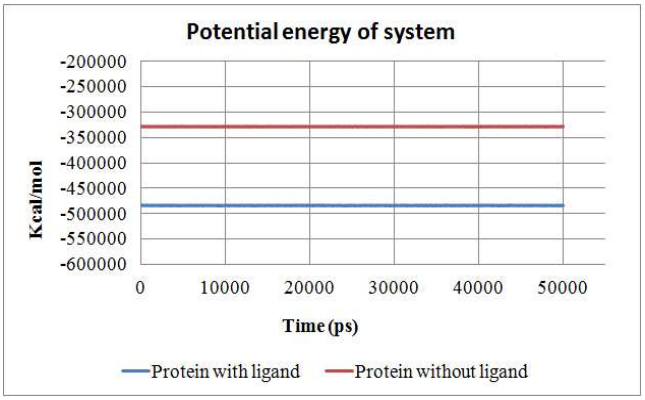


Fig. 4. System potential energy of the LasR dimers throughout simulation.

After simulation we analyzed the system potential energy of the LasR protein in presence and absence of ligand. The average potential energy of the protein dimer bound to its ligand was found to be - 484156.84 Kcal/mol, and the average potential energy of the protein dimer in absence of ligand was found to be -327935.35 Kcal/mol. A lower potential energy value was a reference to a stable system. Energetically, the LasR dimer bound to its ligand (OdDHL) was more stable than the dimer with no ligand bound to it. A graphical representation (Figure 4) of this system potential energy also showed that the LasR dimer with ligand appeared to be more stable throughout the simulation time than the LasR dimer without ligand.

Comparative structural analysis of the dimers

We docked the simulated structure of LasR dimer bound to its ligand (OdDHL), with the promoter DNA (lux α). For a trial we are trying to dock the simulated structure of LasR dimer having no bound ligand with the promoter DNA (lux α). But this trial was not successful. This led us to for a comparative structural analysis. From this analysis we found that the C-terminal domain which was responsible for the DNA binding, of each monomer, of the LasR dimer without ligand, was very close to each other with respect to the LasR protein with ligand. From this analysis it was revealed that the LasR dimer with ligand to form a groovy conformation in the C-terminal region that would help the protein to fit onto the promoter DNA. But the LasR dimer without ligand failed to form this conformation that would make this dimer unable to fit onto the promoter DNA.

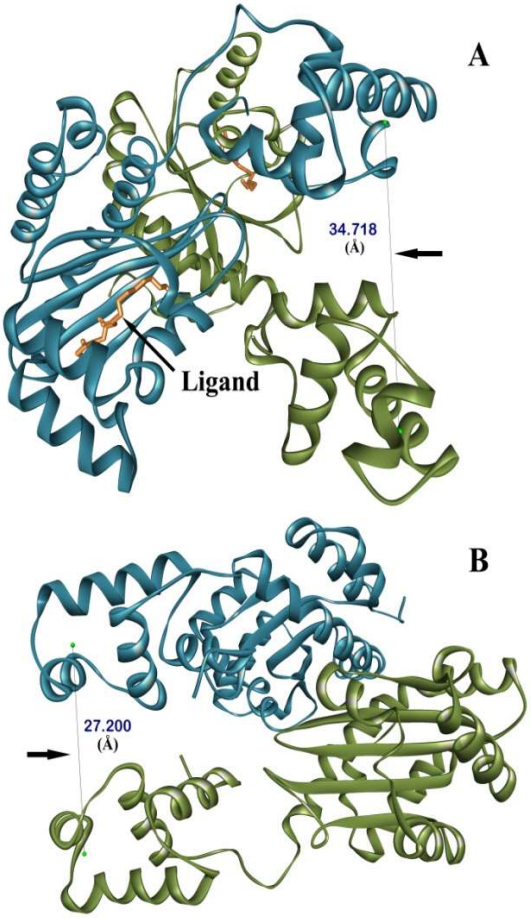


Fig. 5. A) LasR dimer with ligand with a perfect groovy conformation in the C-terminal DNA binding domain. B) LasR dimer without ligand with a comparatively narrow groovy conformation in the C-terminal domain.

We measured the approximate distance between the centeroid points of the HTH motif of the monomers, for the two dimers (Figure 5). For the LasR dimer with ligand the distance is 34.718 Å, and the LasR dimer without ligand the distance is 27.20 Å. And in this two simulated system there was only a single difference, one have ligand another have no ligand. Figure 6 showing a perfect docking of lasR dimer with the promoter DNA in the present of ligand (OdDHL).

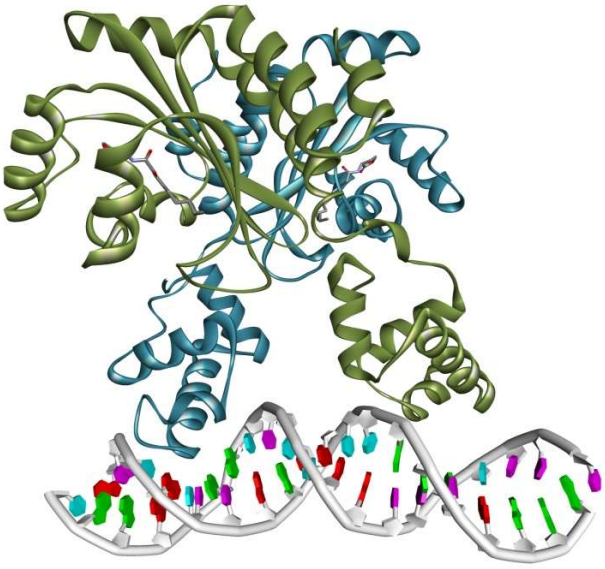


Fig. 6. LasR dimer with the ligand bound with the promoter DNA.

CONCLUSION

LasR transcription factor is very essential for the toxic HCN synthesis in *P. aeruginosa*. From this experiment we found the mode of action of LasR during the transcription regulation of hcnABC genes. The experimental data is the vital evidence for the establishment of the conclusion, that the ligand (OdDHL) is very crucial for the transcriptional activity of LasR. From RMSD to RMSF to System potential energy, all the data indicating that only in the presence of ligand the LasR dimeric system is more stable. And only in the presence of ligand the two C-terminal domain was able to form a special groovy conformation to fit the dimer onto the promoter DNA. So if we could netralize the ligand, we can prevent the LasR transcriptional activity.

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