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Original Article

Theoretical Models of Quorum Sensing Dependent Regulation of Transcriptional Activators of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a successful opportunistic human pathogen which has an ability to secrete numerous virulent factors regulated by quorum sensing. The term quorum sensing, allows a population of bacteria to coordinately control the gene expression of the entire community. Current research on *Pseudomonas aeruginosa* quorum sensing systems confirm that multiple genes were controlled by quorum sensing and many of these were found to be involved in virulence. Furthur it is also becoming clear that quorum sensing systems are under the control of a complex regulatory cascade. The present study was designed to determine the 3D structures and functional analysis of the six transcriptional activators, *Gac A, Las A, Las B, Las R, Rhl R and Vfr* of *Pseudomonas aeruginosa* regulated by quorum sensing mechanism. For each transcriptional regulators 3D models were constructed using homology modeling and validated. The resultant modeled 3D structures of transcriptional activators controlled by quorum-sensing pathways represent a novel point of intervention for the development of antibiotics.

Keywords: Quorum Sensing, Transcriptional Activators, *Pseudomonas aeruginosa*, Homology modeling.

Introduction

Several Gram-negative bacteria use chemical signals to monitor their own species population density and control expression of specific genes in response to population density (De Kievit, and Iglewski, 2000). This process of regulation is termed as quorum sensing (Bassler, 1999). *Pseudomonas aeruginosa* is an opportunistic human pathogen that infects immune-compromised individuals and people with cystic fibrosis (Taylor *et al.,* 1993). Genetic studies of *Pseudomonas aeruginosa* have revealed its two known quorum sensing systems (Chugani *et al.,* 2001; Parsek, and Greenberg, 1999). The first is that of the *las* system, while the second is the *rhl* system. Each system has a transcriptional activator and an autoinducer synthase (Brint *et al.,* 1995). The autoinducer is a diffusible molecule produced at a basal level during low cell density and at higher concentrations as the cell density increases (Parsek *et al.,* 1999). The *P. aeruginosa* autoinducers (PAI-1 and PAI-2) bind to specific target proteins, the transcriptional activators, and these complexes activate a wide number of virulence factors (Holden, 1999). The *las* system consists of the transcriptional activator, LasR (Gambello, and Iglewski, 1991). This protein binds to the autoinducer, N-(3-oxododecanoyl)-L-homoserine lactone (PAI-1) at high cell density and regulates the expression of lasA (LasA protease), apr (alkaline protease A), toxA (exotoxin A), lasI (the PAI-1 synthase), and lasB (elastase). The lasI gene is located immediately downstream of *lasR*. It directs the synthesis of PAI-1. Activation of *lasI* by LasR creates a positive autoregulatory loop. The activation of *rhlR* by LasR results in a quorum-sensing regulatory

cascade, in which activation of the *rhl* system requires an active *las* system (Latifi, 1996; Pesci *et al.*, 1997). The *rhl* system consists of the transcriptional activator, RhlR and of an autoinducer synthase, RhlI. RhlI directs the synthesis of N-butyoyl-1-homoserine lactone (PAI-2). PAI-2 binds to RhlR and this complex activates the transcription of *rhlI*, *rhlA and rhlB*, an operon coding for rhamnosyltransferase, which is required for rhamnolipid production, and *rpoS*, a stationary-phase sigma factor (Ochsner, and Reiser, 1995). There are other control elements that affect the quorum sensing regulatory circuit. The *gacA* gene product is a transcriptional activator that among other things induces C4-HSL production. *Vfr* is a global regulator that affects a mild activation of *lasR*. LasR, which is regulated via *Vfr* and *GacA*, activates *lasI* expression to generate 3-oxo-C12-HSL. This LasR protein–AHL complex positively drives the expression of multiple target genes together with the negative regulator, *LasR*, a second *rhlR* and the genes required for the synthesis of the PQS signal molecule (Pearson *et al.*, 1997). 3-oxo-C12-HSL has both immunomodulatory and vasorelaxant activity and may therefore function as a virulence determinant. Hence, a strategy was designed to model the 3D structures of the transcriptional activators that control the quorum sensing mechanism.

Materials and Methods

The various databases and data analysis tools used in this study are briefly outlined below. KEGG integrates the genomic information with pathways and allows searching for genes and proteins.PDB is a repository for the structural data of biological molecules such as proteins and nucleic acids. SWISS- MODEL is an automated homology modeling server, accessible via the exPASy web server or from the program Deep View. This server is used to make protein modeling accessible to all molecular biologists worldwide (Arnold *et al.*, 2003). PROCHECK is used to check the stereochemical quality of protein structures (Ramachandran plot). Rasmol is a computer program for molecular visualization and to interactively displays the molecule on the screen in a variety of representations with color schemes. The six sequences of transcriptional activators that control the quorum sensing mechanism were retrieved from KEGG database and their annotation were downloaded from SwissProt. The structures for transcriptional activators *Gac A, Las A, Las B, Las R, Rhl R and Vfr* were modeled by subjecting the raw sequence to the Swiss Model Workspace (Arjunan *et al.*, 2010). The swiss model workspace employs the homology modeling for deducing the protein 3D structures. The steps carried to model the 3D structure by Swiss Model workspace is breifly outlined below:

Retrieval of sequences: Sequences of selected transcriptional activators of *Pseudomonas aeruginosa* such as *Gac A, Las A, Las B, Las R, Rhl R and Vfr* were down loaded from KEGG data base.

Finding the templates: An account was created in Swiss model work space. Using option Template identification, Templates with PDB accession number were selected for modeling. The query sequence was pasted and submitted. The obtained templates with high percent identity were taken for homology modeling.

Modelling: The target transcriptional activators (*Las A, Las B, Gac A, Las R, Rhl R and Vfr*) were modeled at SWISS MODEL WORKSPACE by using the obtained templates respectively (*3cz5D, 3it5A, 1u4gA, 3ix4H, 2avxA and 20z6A*) (Abilash *et al.*, 2009). If the identity between the sequence of interest and template structure falls below 40%, the structure does not act as a good model.

Qualifying the model: The Model Quality was assessed by Ramachandran plot using Procheck server where problematic residues are identified by the amino acid residues that have illegal angles.

Results and Discussion

Quorum-sensing systems are believed to be involved in the interactions between many pathogenic bacteria and their hosts (De Kievit, and Iglewski, 2000). Consequently, numerous approaches have been explored aiming to interfere with these signaling processes, including autoinducer- degrading enzymes, inhibition of the autoinducer biosynthetic pathway, and use of autoinducer structural analogs. Few studies have examined directly whether quorum-sensing systems are active in human infections (Miller, and Bassler, 2001). This promoted us to examine the structure and function of the transcriptional

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activators and virulence factors (Whiteley *et al.*, 1999). The first cell-to-cell signaling *las* system consists of the transcriptional activator, LasR, Figure 7 shows the modeled structure of Las R using *3ix4H* as a template and the model quality is of 96 % (Figure 8). This protein binds to the autoinducer, N-(3-oxododecanoyl)-L-homoserine lactone (PAI-1) at high cell density and regulates the expression of lasA and lasB (Chugani *et al.*, 2001; Parsek, and Greenberg, 1999). The modeled structures of this were depicted in the Figure 3 and Figure 5 respectively. These structures were obtained by aligning them to *3it5A* and *1u4gA* templates respectively. The Structural quality of these models was found to be 100 % and 99.6% respectively (Figure 4 & Figure 6). The *las* cell-to-cell signaling system is composed of *lasI*, the autoinducer synthase gene responsible for the synthesis of 3-oxo-C12-HSL (*N*-[3-oxododecanoyl]-L-homoserine lactone, previously named PAI-1, and the *lasR* gene that codes for a transcriptional activator protein. The *las* cell-to-cell signaling system regulates *lasB* expression and is required for optimal production of other extracellular virulence factors such as *LasA*, *Alkaline protease* and *exotoxin A*. *LasI* is the most sensitive gene activated by LasR/3-oxo-C12-HSL. The preference for the *lasI* promoter allows an initial rapid rise in autoinducer synthesis, which increases the amount of 3-oxo-C12-HSL available to bind to LasR to regulate the synthesis of virulence factors *Pyocyanin* and *Superoxidedismutase (*Hassett *et al.*, 1999). The literature survey reveals that autoinduction hierarchy is responsible for a dramatic increase of expression of virulence genes (such as *lasB*). The *las* cell-to-cell signaling system is positively controlled by GacA, as well as by Vfr, which is required for the transcription of *lasR*. The structures of the transcriptional activators Gac A and Vfr were modeled using *3cz5D & 20z6A* by swiss-model workspace and the modeled structures were given in Figure 1 & Figure 11 respectively, and the quality of the structures were found to be 99.5 % and 96.4% respectively (Figure 2 & Figure 12).The multiple regulatory levels of the *las* cell-to-cell signaling system and the various genes under its control highlight the importance of this system for *Pseudomonas aeruginosa*.

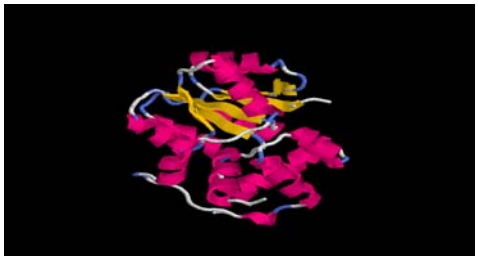

Fig.1: Modeled Structure of Gac A from *Pseudomonas aueroginosa*

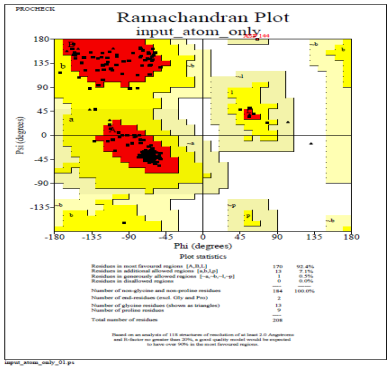

Fig.2: Ramachandran Plot for Gac A

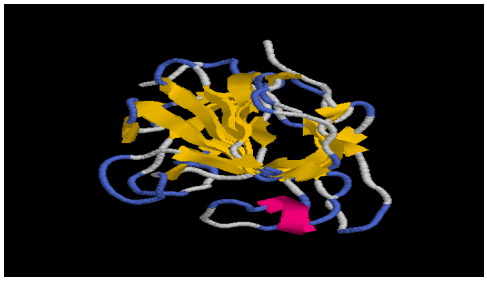

Fig.3: Modeled Structure of Las A from *Pseudomonas aueroginosa*

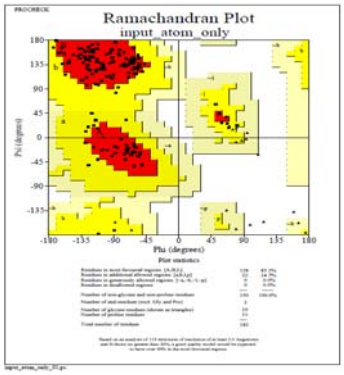

Fig.4: Ramachandran Plot for Las A

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Fig.5: Modeled Structure of Las B from *Pseudomonas aueroginosa*

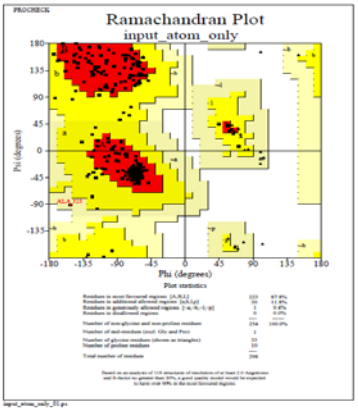

Fig. 6: Ramachandran Plot for Las B

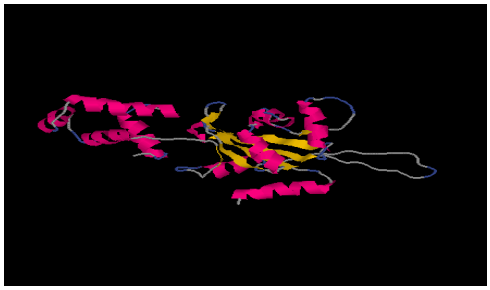

Fig.7: Modeled Structure of Las R from *Pseudomonas aueroginosa*

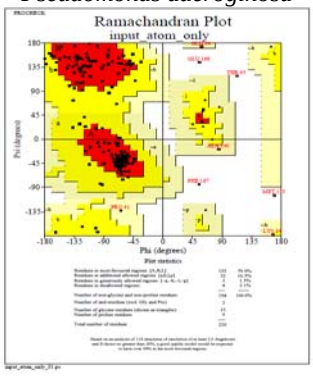

Fig.8: Ramachandran Plot for Las R

Fig.9: Modeled Structure of Rhl R from *Pseudomonas aueroginosa*

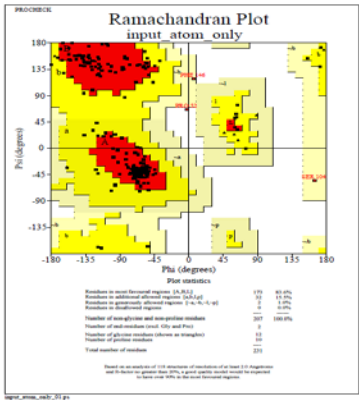

Fig.10: Ramachandran Plot for Rhl R

Fig.11: Modeled Structure of Vfr from *Pseudomonas aueroginosa*

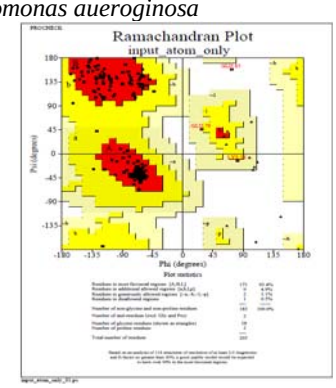

Fig.12: Ramachandran Plot Vfr

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Table -1: Summary of the Quality of the Modeled Transcriptional activators

Sl.No.	Name	No.Amino acids	Amino acids in								No. Of Non Gly & Pro residues	No. Of End residues	No.of Gly residues	No. Of Pro residues
			MFR		AAR		GAR		DAR					
			Residues	Percentage	Residues	Percentage	Residues	Percentage	Residues	Percentage				
1.	Gac A	208	170	92.4%	13	7.1%	1	0.5%	0	0.0%	184	2	13	9
2.	Las A	182	128	85.3%	22	14.7%	0	0.0%	0	0.0%	150	2	19	11
3.	Las B	298	223	87.8%	30	11.8%	1	0.4%	0	0.0%	254	1	33	10
4.	Las R	220	155	79.9%	32	16.5%	3	1.5%	4	2.1%	194	2	15	9
5.	Rhl R	231	173	83.6%	32	15.5%	2	1.0%	0	0.0%	207	2	12	10
6.	Vfr	205	171	93.4%	9	4.9%	2	1.1%	1	0.5%	183	2	18	2

MFR – Most favoured region AAR - Additionaly allowed regions GAR - Generously allowed regions DAR-Disallowed regions

Table -2: Predicted secondary structure of Transcriptional Activators

Sl.No.	Name	Total No.Amino acids	Secondary Structural Elements								
			Alpha Helix (Hh)			Extended Strands (Ee)			Random coils (Cc)		
			Number of Helices	Residues	Percentage	Number of sheets	Residues	Percentage	Number of Coils	Residues	Percentage
1.	Gac A	208	22	95	44.39%	12	32	14.95%	37	87	40.65 %
2.	Las A	182	02	143	34.21%	40	79	18.90%	46	196	46.89 %
3.	Las B	298	11	125	25.20%	19	115	23.19%	34	256	51.61 %
4.	Las R	220	11	99	41.42%	08	33	13.81%	20	107	44.77 %
5.	Rhl R	231	13	85	35.42%	10	32	13.33%	21	123	51.25 %
6.	Vfr	205	12	106	49.53%	34	34	15.89%	35	74	34.58 %

Table -3: Structural informations of the Modeled Transcriptional Activators

Sl.No.	Name	Structural informations			Chains	Groups	Atoms	Bonds	Hbonds
		Helices	Strands	Turns					
1.	Gac A	22	12	37	2	413	3196	6165	289
2.	Las A	2	40	46	2	364	2825	5842	211
3.	Las B	11	19	34	1	298	2318	2380	205
4.	Las R	11	8	20	1	220	1730	1773	135
5.	Rhl R	13	10	21	1	231	1863	1907	141
6.	Vfr	12	34	35	2	406	3222	6486	310

Pseudomonas aeruginosa has a second cell-to-cell signaling system, named the *rhl* system because of its ability to control the production of rhamnolipid (Ochsner, and Reiser, 1995). This system is composed of *rhlI*, the C4-HSL autoinducer synthase gene, and the *rhlR* gene, the structure of this was modeled using template *2avxA* and depicted in Figure 9 and the model is of 98.3% when checked with ramachandran plot(Figure 10) encoding a transcriptional activator protein. The quality of the modeled structures, the secondary structure and the structural informations were summarized in the Table 1, 2 &3 respectively. Thus the modeled 3D structures of transcriptional activators controlled by Quorum-sensing pathways represent a novel point of intervention for the development of antibiotics.

Quorum sensing is employed by bacteria to detect the presence and number of other bacteria in their environment and a bacterium responds to this information by altering patterns of gene expression. Many Pathogens do not express virulence factors before reaching high density, presumably to avoid alerting the host until the bacteria numbers are large enough to overcome an immune response. Therefore, quorum-sensing pathways represent a novel point of intervention for the development of antibiotics. One quorum-sensing pathway, signaling system 2, is utilized by a wide variety of bacteria and appears to be a nonself-specific method of sensing environmental cell density. This system may provide a target for novel broad-spectrum antibacterial agents. System 2 is relatively poorly understood. From genetic studies, Las R is known to be required for AI-2 generation but its specific biochemical role is unclear, and no other components of the AI-2 biosynthesis pathway have been described. This paper reports the structure and functional analysis of the transcriptional activators Gac *A, Las A, Las B, Las R, Rhl R and Vfr* of *Pseudomonas aeruginosa*. These provide the ground- work for mutagenesis experiments and also, drug candidates can now be explored that will interfere with AI-2 production and act as a multispecies antibiotic.

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