Antiquorum sensing activity of silver nanoparticles in *P. aeruginosa*: an in silico study

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Abstract *Pseudomonas aeruginosa* an opportunistic pathogen regulates its virulence through Quorum sensing (QS) mechanism comprising of Las and Rhl system. Targeting of QS mechanism could be an ideal strategy to combat infection caused by *P. aeruginosa*. Silver nanoparticles (AgNPs) have been broadly applied as antimicrobial agents against a number of pathogenic bacterial and fungal strains, but have not been reported as an anti-QS agent. Therefore, the aim of present work was to show the computational analysis for the interaction of AgNPs with the QS system using an In silico approach. In silico studies showed that AgNPs got ‘locked’ deeply into the active site of respective proteins with their surrounding residues. The molecular docking analysis clearly demonstrated that AgNPs got bound to the catalytic

cleft of LasI synthase (Asp73-Ag = 3.1 Å), RhII synthase (His52-Ag = 2.8 Å), transcriptional receptor protein LasR (Leu159-Ag = 2.3 Å) and RhlR (Trp10-Ag = 3.1 Å and Glu34-Ag = 3.2 Å). The inhibition of LasI/RhII synthase by AgNPs blocked the biosynthesis of AHLs, thus no AHL produced, no QS occurred. Further, interference with transcriptional regulatory proteins led to the inactivation of LasR/RhlR system that finally blocked the expression of QS-controlled virulence genes. Our findings clearly demonstrate the anti-QS property of AgNPs in *P. aeruginosa* which could be an alternative approach to the use of traditional antibiotics for the treatment of *P. aeruginosa* infection.

Keywords In silico · Molecular docking · Quorum sensing · *Pseudomonas aeruginosa* · Virulence · Silver nanoparticles

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Introduction

Quorum sensing (QS) is a phenomenon of a cell to cell communication using diffusible chemical signaling molecules (Reading and Sperandio 2006; Banik et al. 2009). QS molecules control an array of pathogenic behavior such as the production of virulence factors and biofilm formation (Davies 2003) in many gram negative and gram positive bacteria. *Pseudomonas aeruginosa* is opportunistic pathogens whose most of the virulence characters are QS-controlled and cause acute infection in respiratory systems, particularly in cystic fibrosis and HIV patients (Palmer et al. 2005). However, treatment with antibiotics makes the antibiotic treatment ineffective as it develops resistance within a short period of time (Su et al. 2010; Juan et al. 2010). *P. aeruginosa* controls its pathogenicity by producing, detecting and responding to extracellular signaling molecules called as

autoinducers (Longo et al. 2013). Studies regarding Quorum sensing signalling molecules (QSSMS) or autoinducers in *P. aeruginosa* have confirmed that these autoinducers in *P. aeruginosa* are called as acyl homoserine lactones (AHLs) which control the production of virulence factors, antibiotic resistance and biofilm formation (Pearson et al. 1997; Delden and Iglewski 1998; Pesci et al. 1999). The two QS-systems which are involved in the pathogenicity of *P. aeruginosa* using autoinducers are Las and Rhl systems. The Las QS system through its LasI gene synthesizes N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) which is detected by its homolog LasR and Rhl system through its RhlI gene synthesize N-butyl-L-homoserine lactone (C4-HSL), which is recognized by its receptor RhlR. When a certain threshold concentration of 3-oxo-C12-HSL is reached, it binds to the receptor protein LasR and activates the expression of virulence genes as proteases, elastases and exotoxin A (Passador et al. 1993). In addition, the LasI homolog RhlI is regulated by LasR-3-oxo-C12HSL complex. RhlI after the threshold level of C4-HSL binds to the receptor RhlR and activates target genes, responsible for the production of pyocyanin, elastases, siderophores, and rhamnolipids (Wagner et al. 2008). The combined effect of QS complex leads to the infection although antibiotics are available against the infection but the development of antibiotic resistance makes this bacterium more harmful. Therefore, there is an urgent need to search for the cost-effective and alternative remedy for the treatment of *P. aeruginosa* infection. Interfering with these bacterial QS systems could have broad application in biological control of disease since these QS molecules are used to regulate virulence (Alvarez et al. 2012). Nanotechnology could provide an answer to this where small particles of nano size called as nanoparticles could interfere with these QS molecules. Nanoparticles have many advantages like reduce toxicity which overcomes resistance and are also cost effective (Pal et al. 2007; Weir et al. 2008). It has been proved that naturally occurring bacteria do not develop resistance to nanoparticles (Mühling et al. 2009). Some of the previous reports have shown the down regulation of QS mediated virulence factors by AgNPs (Singh et al. 2015; Ali et al. 2017). Vyshnava et al. (2016) have also shown the antibiofilm potential of AgNPs using In silico approach. Most of the reports claiming AgNPs to be anti-QS agents are available but very few reports have focused onto to the computational analysis of Ag with QS. Keeping in view about the importance of computational analysis and limited availability of literature about AgNPs and QS controlled virulence genes we have designed few objectives as (1) In silico study of anti-QS activity of AgNPs, (2) molecular docking analysis of interaction of AgNPs with QS-controlled proteins LasI/RhlI AHL synthase, (3) molecular docking analysis of interaction of AgNPs with QS-controlled transcriptional regulatory proteins LasR/RhlR and virulence genes.

Materials and methods

In silico analysis

Preparation of Las synthase and Las receptor molecules

The 3D structure of LasI Synthase (PDB ID: 1RO5) and transcriptional activator protein LasR (PDB ID: 2UV0) was retrieved from Protein Data Bank (www.rcsb.org). Unfortunately, the crystal 3D structures of RhlI Synthase and regulatory protein RhlR were not available in the Protein Databank, so, we have modelled the 3D structure of RhlI Synthase and RhlR using SWISS-MODEL homology method. PROMOD-II tool integrated into SWISS-MODEL has been used in the model building on the basis of target-template alignment (Sali and Blundell 1993; Guex et al. 2009). QMEAN scoring function has been used by SWISS-MODEL to estimate the overall quality of modelled 3D structure (Benkert et al. 2011). All the water molecules and hetero atoms were removed. The Model validation analysis was completed by RAMGAGE Ramachandran Plot analysis server (<http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>) (Lovell et al. 2002).

Preparation of 3D structure of Ag

The .mol file of Silver (Ag) was taken from Protein Data Bank and converted into .pdb files using Open Babel software. Discovery Studio makes it easier to examine the properties of large and small molecules. Gasteiger charge was added to the ligand. Further, the ligand was submitted for minimization using Chimera version 1.10 using Genetic Algorithm Steps 2000 and 0.5 grid units Optimized (Pettersen et al. 2011).

Prediction of docking/active site of Las and Rhl systems

Prediction of the Ag binding sites for generated LasI/R and RhlI/R models was done by the 3D Ligand site developed by the structural bioinformatics groups, Imperial College London. The pdb files were uploaded in the space provided in the tool, and these pdb files were processed for the identification of active binding sites for Ag ligands based on the critical assessment of techniques for protein structure prediction (Wass et al. 2010).

Molecular docking

Docking was performed over rigid protein structures of LasI Synthase, LasR receptor, RhlI Synthase and RhlR receptor. The interaction of AgNPs with AHL Synthase LasI, LasR receptor, AHL Synthase RhlI and RhlR receptor was explored through PatchDock tool (Schneidman-Duhovny

et al. 2005). PatchDock is a geometry-based molecular docking algorithm which is Critical Assessment of Prediction of Interactions (CAPRI). The default RMSD value for this parameter was 4 Å. Based on the ranking, the scores of the docked file were selected and subjected to the post-docking 3D simulation using discovery studio visualize 4.5 and PyMol 3D visualization software.

Results

In silico studies

The 3D crystal structure of LasI Synthase (PDB ID: 1RO5; Fig. 1a) and transcriptional activator protein LasR (PDB ID: 2UV0; Fig. 2a) was retrieved from Protein Data Bank. Due to the unavailability of a 3D crystal structure of RhII Synthase and regulatory protein in the Protein Databank, we modelled the 3D structure of RhII Synthase (Fig. 3a) and RhIR (Fig. 4a) using SWISS-MODEL homology method. The Model validation of conformation of RhII Synthase and

RhIR was done by Ramachandran Plot (Hollingsworth and Karplus 2010) based on the Chi (Φ) and Psi (Ψ) analysis; Ramachandran Plot of RhII Synthase showed 94.5% of the residues in favored region, 4.9% residues in the allowed regions and 0.5% residues in outlier region (Fig. 3b). Similarly, Ramachandran Plot of RhIR showed 97.2% of the residues in the favored region, 2.1% residues in the allowed regions and 0.6% residues in outlier region (Fig. 4b).

The molecular docking results showed that AgNPs were docked into AHLs synthase LasI (Fig. 1b) and RhII (Fig. 3c) as well as regulatory activator proteins LasR (Fig. 2b) and RhIR (Fig. 4c) successfully. Based on this docking pose, we speculated that the AgNPs got “locked” deeply into the active site of respective proteins with their surrounding residues (Figs. 1c, 2c, 3d, 4d). The molecular docking analysis showed that the Ag(0) got bound to the catalytic Aspartate residue of synthase LasI (Asp73-Ag = 3.1 Å; Fig. 1c), Leucine residue of LasR receptor (Leu159-Ag = 2.3 Å; Fig. 2c), Histidine residue of RhII synthase (His52-Ag = 2.8 Å; Fig. 3d) and Tryptophan (Trp10-Ag = 3.1 Å) and Glutamate (Glu34-Ag = 3.2 Å) residues of RhIR receptor (Fig. 4d).

Fig. 1 3D structure of Acyl-homoserine lactone (AHL) Synthase LasI (a); binding of AgNPs with the active site of LasI (b); close view of catalytic site of LasI, with bound Ag represented as blue sphere. Amino acids residues (Asp73) of LasI involved in the interaction with AgNPs (c). LasI active site has been depicted by PyMol viewer. Interaction analysis of AgNPs with LasI has been explored through PatchDock

Fig. 2 3D structure of transcriptional activator protein LasR (a); binding of AgNPs with the active site of LasR (b); close view of catalytic site of LasR, with bound Ag represented as blue sphere. Amino acids residues (Leu159) of LasR involved in the interaction with AgNPs (c). LasR active site has been depicted by PyMol viewer. Interaction analysis of AgNPs with LasR has been explored through PatchDock

Discussion

The molecular docking is a powerful computational based approach that is used to model the interaction between a small molecule and a protein at the atomic level to characterize and elucidate the behavior of small molecules in the binding site of target proteins. We were therefore interested in carrying out exploratory studies into the QS-modulatory activity of AgNPs by In silico approach. It is well known that *P. aeruginosa* utilizes QS by producing AHLs molecules to control numbers of virulence factors. For example, the Las and Rhl system regulates the production of the virulence factors protease, elastases, exotoxin A, pyocyanin, rhamnolipid, hydrogen cyanide and biofilms formation (Hodgkinson et al. 2012). Thus, interfering with AHL-based QS by AgNPs is expected to become a master plan to attenuate the virulence in *P. aeruginosa* and can be utilized as a novel chemotherapeutic agent to overcome the QS-controlled infections. The LasI and RhlI synthase direct the synthesis of 3-oxo-C12-HSL and C4-HSL, respectively in *P. aeruginosa* which

triggers the LasR and RhlR-encoded transcriptional activator (LasR and RhlR) to induce the virulence genes. Therefore, Las and Rhl systems were considered as a molecular target for AgNPs. To understand the mechanism of AgNPs mediated inhibition of AHL synthase (LasI/RhlI) and suppression of transcriptional regulatory receptors proteins (LasR/RhlR); molecular docking analysis was performed to predict the probable AgNPs binding site in AHL synthase and LasR/RhlR receptors.

The Model validation of conformation of RhlI Synthase and RhlR was done by Ramachandran Plot based on the inspection of Chi (Φ) and Psi (Ψ). Our data of Ramachandran Plot of RhlR are almost similar to the previous report of Gnanendra et al. (2010). Modelled 3D structures of LasI/R and RhlI/R was submitted to minimization process. Chimera 1.10 was used for energy minimization, removal of steric collision with the steepest descent steps 1000, steepest descent size 0.02 Å, conjugated gradient steps 1000 and the conjugate gradient step size 0.02 Å for the conjugate gradient minimization (Pettersen et al. 2011; Wang et al. 2004,

Fig. 3 3D structure of Acyl-homoserine lactone (AHL) Synthase RhlI (a); RAMPAGE validation of conformation of RhlI (b); binding of AgNPs with the active site of RhlI (c); close view of catalytic site of RhlI, with bound Ag represented as blue sphere. Amino acids resi-

dues (His52) of RhlI involved in the interaction with AgNPs (d). RhlI active site has been depicted by PyMol viewer. Interaction analysis of AgNPs with RhlI has been explored through PatchDock

2006). Our data on molecular docking analysis (Figs. 1, 2, 3, 4) clearly shows that silver binds in the catalytic cleft of AHLs synthase (LasI/RhlI) receptor proteins (LasR/RhlR) and finally, inhibits the QS-controlled virulence genes and biofilm formation in *P. aeruginosa*. Our molecular docking results on the interaction and bindings of Ag with LasR and RhlR are consistent with the findings of Vyshnav et al. (2016). Although the interaction and binding of AgNPs with LasI and RhlI has not been previously reported in the literature, herein for the first time, we have shown the binding of AgNPs with the LasI (Fig. 1) and RhlI signal synthase (Fig. 3).

Based on In silico findings, we have suggested following possible mechanisms of AgNPs interruption with QS circuits, including (1) blocking of biosynthesis of S-adenosyl methionine (SAM), SAM acts as an amino donor for generation of the homoserine lactone ring moiety. It is supposed that AgNPs may bind to SAM, which leads to depletion of SAM and finally no AHL produced (Fig. 5-1). (2) Inhibition of signal biosynthesis i.e., inhibition LasI/RhlI synthase.

The AgNPs may bind to AHL synthase, thereby inhibiting the enzymatic activity. Thus, if no AHL produced, no quorum sensing occurs (Fig. 5-2). (3) Interference with signal receptor proteins LasR and RhlR. When the bacterial cell density reaches a particular threshold, the LasI/R QS system gets initiated. Ag binds with the active site of LasR. The Ag-LasR complex so formed inhibits the expression of RhlI as well as LasR-controlled genes i.e., LasI signal synthase, which then leads to inactivation of the RhlI/R system that finally blocks and inhibit the expression of QS-controlled virulence genes (Fig. 5-3).

Conclusion

It has been concluded that jamming bacterial cell-to-cell communication could be a master plan in developing a novel chemotherapeutic agents. To the best of our knowledge, this is the first In-silico report showing that AgNPs can inhibit AHLs production by interfering with AHL Synthase LasI/

Fig. 4 3D structure of regulatory protein RhIR (a); RAMPAGE validation of conformation of RhIR (b); binding of AgNPs with the active site of RhIR (c); close view of catalytic site of RhIR, with bound Ag represented as blue sphere. Specific residues (i.e., Trp10

& Glu34) of RhIR involved in the interaction with AgNPs (d). RhIR active site has been depicted by PyMol and the interaction analysis of AgNPs with RhIR has been explored by PatchDock

Fig. 5 Schematic representation of anti-QS activity of green synthesized AgNPs

RhII and regulatory receptor proteins LasR/RhIR. Interestingly, AgNP's have no chemical structure similarity to AHLs analogues, which again suggests a novel mechanism of inhibition of QS-controlled genes and can be an alternative to treat *P. aeruginosa* infections. The molecular docking analysis

result showed that AgNPs got bound to the active site of LasI (Asp73)/RhII (His52) and LasR (Leu159)/RhIR (Trp10 and Glu34) of the proteins. Thus, the inhibition of quorum sensing in *P. aeruginosa* using nanoparticles can be recognized as an alternative approach to the use of traditional antibiotics for the treatment of infections caused by this organism. Overall, In silico findings, reveals that the AgNPs can combat with bacterial infections and can act as a novel target for inhibition of QS mediated virulence in *P. aeruginosa*.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animals performed by any of the authors.

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