Use of computational methods in the studies of bacterial proteins TraR and AiiO

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Abstract

Bacteria have developed adaptation mechanisms allowing them to stand against the conditions of ecological niche they are present in. Quorum sensing is one of such mechanisms. It regulates expression of genes in bacterial cells and depends on density of signal molecules produced by those cells. Binding of signal molecules to their receptors leads to activation of particular genes which are responsible for changes of phenotype such as production of virulence factors or synthesis of molecules needed chemiluminescence reactions. LuxI/LuxR system is the most common quorum sensing mechanism and it utilizes homoserine lactones (AHL) as a signal molecules.

It has been confirmed that processes that lead to blocking of activity of quorum sensing system (and expression of genes controlled by this system) exist. This can be caused by receptor inhibitors, the molecules which structures allow to bind strongly with binding site. Once inhibitor is bound to the receptor, AHL molecules have no such possibility and activation of genes regulated by quorum sensing systme is blocked. During my research I have been studying if molecules of plumbagin and 3-chlorideplumbagin can bind to binding site of TraR receptor of *Agrobacterium tumefaciens*. TraR is part of TraI/TraR quorum sensing system which is analogue to LuxI/LuxR system utilizes OOHL molecules (which are homoserine lactone). Binding of OOHL leads to dimerisation of TraR protein that allows dimer to strongly bind specific site on DNA called *tra* box and as a result activates transcription of

genes responsible for the transfer of Ti plasmid. Ti plasmid can transfect plant cells. It contains DNA that can cause tumors on plant roots. I used molecular docking to calculate average binding energies of plumbagin and 3-chlorideplumbagin to TraR protein ligand binding site and DNA binding site on the surface. By use of this method I was also able to create models of their binding to TraR protein that may result in blocking its function.

My second study focused on other possibility of blocking quorum sensing mechanism which is called quorum quenching. Quorum quenching depends on hydrolysis of bonds of signal molecules by specific enzymes. The enzyme I have been studying is a protein AiiO from *Ochrobactrum* sp., which has been reported to hydrolize acyl bond of AHL molecules, therefore it has been classified as acylase. When I started my research the structure of this protein has not been known yet. I have predicted structure of AiiO using aminoacid sequence with I-TASSER software. I classified this protein asα/β hydrolase with Ser-Glu-His catalytic triad. I've located enzyme active site with MetaPocket method and it contained mentioned catalytic triad. In the meantime the crystal structure of AiiO has been published and my comparison of both structures confirmed that model created by me was accurate. The same publication classified the enzyme as a lactonase though. It reported that AiiO have hydrolised bonds of lactone ring. I have decided to study both hydrolysis mechanisms and enzyme interactions with AHL molecules. I have used Water-Swap procedure to determine free binding energy of AiiO complexes with chosen AHL molecules and aminoacid contributions to their binding. Molecular dockings helped me to estimate average binding energies of AHL molecules to the active site. I chose AHL moleucles that differ by their structure (carbon chain lenght and substitute in position 3.). I have observed that those molecules had two significant binding conformations which exposed either acyl group or lactone ring to the catalytic triad. This suggested that it is possible that enzyme catalyzes two types of hydrolysis depending on binding mode of a substrate. I used HHL and OdDHL molecules, reported to be hydrolised by AiiO with the highest accuracy, as a ligands of AiiO in molecular dynamics simulations performed by AMBER12. The results showed the dynamics

of the molecules inside active site and their interaction with protein. Basing on trajectories from molecular dynamics I have prepared systems for QM/MM studies of two possible mechanisms of reactions- hydrolysis catalysed by acylase and catalysed by lactonase which included proton transfer within catalytic triad and nucleophilic attack on proper atom of carbon. QM/MM method of simulations joins molecular mechanics of the whole complex with quantum mechanics of its chosen atoms. Such approach allows to study forced transitions of atoms and process of bonds being created or broken. Thanks to this method I was able to determine that it is unlikely that enzyme will catalyze hydrolysis within acyl group of AHL molecules because it requires incomparably big energies in comparison to hydrolysis within lactone ring. It is caused by lack of suitable stabilisation of tetraedric intermediate that is created in the simulation.