Research Paper Biotechnology

Stress As Antibiotic Resistance Determinant in Bacteria: A Homology Model Perspective

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ABSTRACT

The hypothesis that bacteria would likely attain resistance to any antibiotic treatment given the right circumstance. Moreover, since antimicrobials are growth inhibiting stressors that often elicit protective responses in bacteria, these agents can provoke their own resistance promoting responses. The universal stress proteins have functional roles in adhesion, motility and oxidative stress resistance. However, some forms are not directly involved in stress resistance but are essential. Here, structural features of universal stress proteins from E.coli and Corynebacteriales bacterium complexed with dioxygen and lactum ring antibiotic Penicillin have been presented through docking and homology modeling approach. The study underlines the effect of oxygen and lactum antibiotic Penicillin on the stress protein derived from both gram + and – bacteria through identified amino acid residues and substrate interactions. The structure models can provide clues to structurally understand the importance of stress responses as resistance determinants and appreciate their value as therapeutic targets.

KEYWORDS : Stress, Antimicrobials, Resistance determinant, Universal stress protein, Homology Modeling

Introduction:

The expanded research in bioactive small molecules mediated cell-cell interactions in microbiology has led to the assumption that most interspecies cell-cell interactions could be termed as communication or signaling. However, it is important to mention that demonstration of a biological response upon exposure to a chemical does not necessarily imply communication. The improper use of terms like signals, signaling or communication in microbiology has created confusion since most interspecies metabolite mediated interactions labeled as communication/signaling are often in conflict with evolutionary theories.[1,2] To determine whether an interaction is mediated by a signal, a cue or coercion the overall benefit of the reaction is used as primary criteria. To first differentiate the biological responses induced by antibiotics, their bioactivities on a large concentration spectrum from the perspective of bacteria is considered. From high to low concentrations antibiotics act as toxins, stress inducer, cues or coercions. [1,2] The study tries to explain further the impact of antibiotic resistance on bacterial response upon exposure to antibiotics. The antimicrobial behavior of antibiotics occurs when their concentration is high leading to bacterial death or arrest of growth in susceptible receiver cells. At lower concentrations (sub inhibitory) antibiotics can act as stress inducer or coercions and be sensed as cues. At sub inhibitory concentrations antibiotics can affect several cellular processes or alter gene expression leading to different adaptive responses affecting antibiotic resistance or tolerance. [1,2]

A signal is defined when both partners get advantage of the interaction (bidirectional), while cues or coercions have unidirectional benefits for receivers or emitters, respectively. Although, not mediated by single molecule, environmental conditions can also be considered as cues by bacteria and they include pH, osmolarity, temperature, oxidative stress/oxygen and nutrient limitation. Antibiotic resistance mechanisms and the antimicrobial nature of antibiotics are often associated as a cause and effect phenomenon.[1,2] It has been widely established that antibiotic resistance determinants have specifically evolved to tolerate the lethal activity of antibiotics, but experimental data to fully support this hypothesis are still lacking. Antibiotics are mainly present at non-lethal concentrations in the environment, thus antibiotic resistance determinants are likely involved in response mechanisms other than those required when receiver bacteria are exposed to lethal concentrations. Similar to the antibiotic concentration dependent response, antibiotic resistance would also impact the receiver bacteria in an antibiotic dose dependent manner. The presence of an antibiotic resistance mechanism would shift the spectrum of responses to an antibiotic to higher concentrations. The resistance would lower the antibiotic concentration at the target site. Exceptions to this would occur if there were secondary target sites for the antibiotics that mediate other responses. At toxic concentrations, resistance would function in the conventional protective role and allow receiver bacteria to avoid the antibiotic toxicity by blocking death or growth arrest. At sub inhibitory concentrations, antibiotic resistance genes would shift the effective antibiotic concentration required for inducing the biological responses (stress inducers, coercions or cues) of receiver bacteria. The displacement of the response curve to sub inhibitory concentrations of antibiotics due to the presence of resistance determinants could therefore establish that stress induction, coercion and detection of cues are selectable phenotypes that can be tuned to meet the conditions of natural environments. Antibiotics at sub inhibitory concentrations can act as stress inducers or cues/coercion on receiver bacteria. When behaving as stress inducers, antibiotics often induce the SOS stress response, which is also associated with various antibiotic resistance mechanisms.[1-4] The following sections include the involvement of universal stress protein and the impact of antibiotic resistance on the antibiotic induced stress responses in both gram positive and negative bacteria. The universal stress protein superfamily encompasses a conserved group of proteins that are found in bacteria, archaea and eukaryotes. Based on structural analysis and their amino acid sequence, the Usp proteins have been divided into different classes. The levels of universal stress proteins become elevated in response to a variety of stress conditions including antibiotics. It has been reported that universal stress proteins have functional roles in adhesion, motility and oxidative stress resistance. However, some forms are not directly involved in stress resistance but are essential. [14]

Materials and Methods:

Sequence search:

Sequences of Universal Stress Proteins, UspC (E. *coli* Nissle 1917, Gram – bacteria) and USP (Corynebacteriales bacterium X1036, Gram + bacteria) were retrieved from the NCBI protein database.

Template search:

Template search with BLAST [5] and HHblits [6] has been performed against the SWISS-MODEL template library.[15] The target sequences were searched with BLAST against the primary amino acid sequence contained in the SMTL. Two templates have been selected, one for UspC from E.*coli* and other for USP from Corynebacteriales bacterium X 1036 among various templates. An initial HHblits has been built using the procedure outlined in the Remmert *et al.,* 2011 method, followed by 1 iteration against NR20. The obtained profile has then been searched against all profiles of the SMTL.

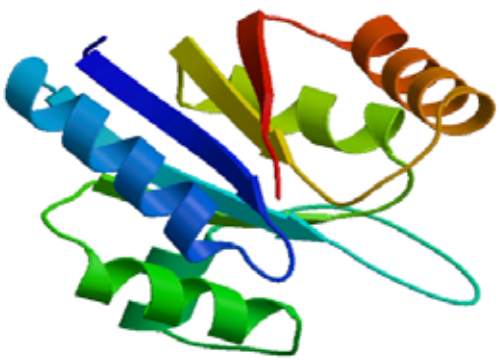
Template selection:

Templates for UspC (E.*coli*) include (1jmv.1.A; 1jmv.1.B; 1jmv.2.A) and for USP (Corynebacteriales bacterium X1036) templates are (2jax.1.A; 3cis.1.A; 3cis.1.B). Based on sequence identity and sequence similarity, 1jmv.1.A (sequence identity 30.15%, sequence similarity 0.36) for UspC (E.*coli*) and 3cis.1.A (sequence identity 33.45%, sequence similarity 0.37) for USP (Corynebacteriales bacterium X1036) were selected as ideal templates for model building. For each identified template, the template quality has been predicted from features of the target-template alignment. The templates with highest quality have

been selected.

Model building:

The models were built based on target-template alignment with ProMod Version 3.70. The models have GMQE value 0.68 and Seq. similarity 0.36 for UspC (E.*coli*) and GMQE value 0.70 and Seq. similarity 0.37 for USP (Corynebacteriales bacterium X1036) respectively. (Fig.1.) Coordinates, which are conserved between the target and the templates are copied from the template to the model. Insertions and deletions are remodeled using a fragment library and side chains are then rebuilt. Finally the geometry of the resulting models is regularized by using a force field. In case loop modeling with ProMod Version 3.70 does not give satisfactory results, an alternative model was built with MODELLER. [7] The global and per- residue model quality was assessed using the QMEAN scoring function.[8]



(a)

(b)

Figure 1. (a) UspC (E.*coli*) (b) USP (Corynebacteriales bacterium X1036)

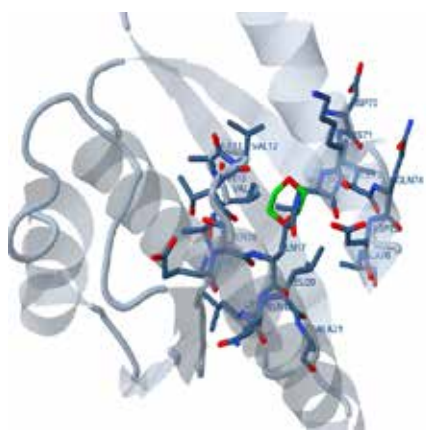
Oligomeric state conservation:

The models have an oligomeric state of Monomer. Homo-oligomeric structure of the target protein is predicted based on the analysis of pairwise interfaces of the identified template structures. For each relevant between polypeptide chains (interfaces with more than 10 residues-residues interactions), the QscoreOligomer is predicted from features such as similarity to target and frequency of observing the interface in the identified templates.[9] The prediction is performed with a random forest regressor using these features as input parameters to predict the probability of conservation for each interface. The oligomeric state of the target is predicted to be the same as in the template when QscoreOligomer is predicted to be higher or equal to 0.5.[9]

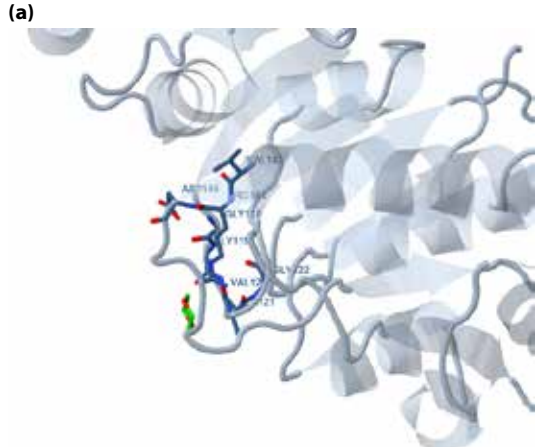
Results:

A significant environmental effect on bacteria is stress, which in terms of affecting a myriad of adaptive and protective responses, alters gene expression patterns and cell physiology in several ways that can influence antimicrobial susceptibility. This occurs indirectly as a result of stress induced growth cessation or dormancy, since antimicrobials typically act on growing cells or directly as a result of stress dependent recruitment of resistance determinants (e.g. antimicrobial flux), changes to antimicrobial targets, amelioration of the adverse consequences of the antimicrobial action, alteration to the membrane barrier functions etc. Moreover, since antimicrobials are growth inhibiting stressors that often elicit protective responses in bacteria, these agents can provoke their own resistance promoting responses. The study highlights a variety of bacterial stress responses especially under oxidative stress that could be linked to antimicrobial resistance, providing support for stress responses themselves being resistance determinants. [1,2]

Further, docking studies were performed to study the effect of oxidation on stress proteins both UspC (E.*coli*) and USP (Corynebacteriales bacterium X1036). Dioxygen was incorporated into the structures of UspC and USP with estimated free energy of binding -3.06 kcal/mol and -1.35 kcal/mol and estimated Inhibition constant Ki value 5.75 mM and 101.65 mM respectively. Docking calculations were carried out using DockingServer.[10] Gasteiger partial charges were added to the ligand atoms.[11] Non-polar hydrogen atoms were merged and rotatable bonds were defined. Docking calculations were carried out on dioxygen protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. [12] Affinity (grid) maps of 0.2 to 1.0 Å grid points and 0.375 Å spacing were generated using the Autogrid program. [12] AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. [13] Initial position, orientation and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.



(a)



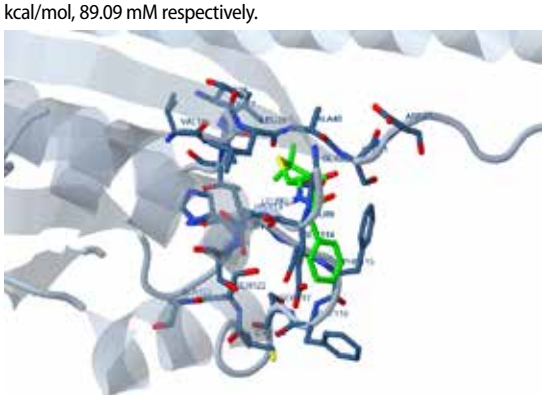
(b)

Figure 2. (a) UspC with dioxygen (b) USP with dioxygen

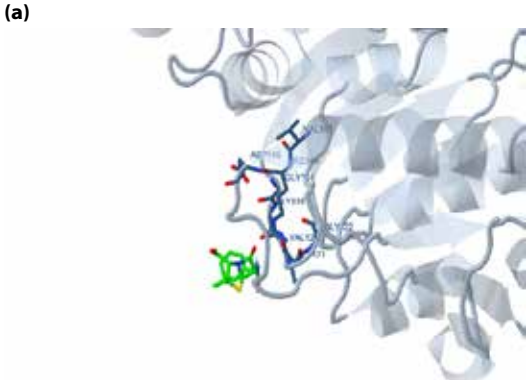
The structure of UspC incorporated with dioxygen suggests that some specific amino acid residues in the UspC are involved in hydrophobic, polar and other types of interactions with oxygen atoms of dioxygen. (Fig.2) The residue Asp75 (decomposed interaction energy 0.0665 kcal/mol) shows polar interaction with the oxygen atom. Some resi-

dues including Leu20 (-0.22), Leu72 (-0.04) and Val10 (-0.08) are involved in hydrophobic interaction. Other types of interactions include Gln17 (-0.30), Lys71 (-0.27) and Ser16 (-0.21) residues. (Fig.2 (a)) In case of USP (Corynebacteriales bacterium X1036) the residue Arg144 (-0.38) is interacting with the oxygen atom of dioxygen. (Fig.2 (b))

Organisms that grow in aerobic conditions are routinely exposed to oxidative stress in the form of reactive oxygen species (ROS) e.g. peroxide, superoxide etc. that are harmful and avoidable by products of aerobic respiration. ROSs damage a variety of cellular macromolecules and thus elicit adaptive oxidative stress responses in bacteria intended to survival in the presence of this stressor.[1,2] Expression of multidrug efflux systems are positively impacted by agents of oxidative stress, these efflux systems possibly play a role in ameliorating the effect of this stress. Similarly, antioxidant mechanisms are recruited in response to antimicrobial exposure, antimicrobials being known to generate ROSs that are often key to the lethal effects of these agents. As such oxidative stress has the potential to antimicrobial resistance in a variety of ways. Humans, animals, and plants are in continuous contact with beneficial, harmless or pathogenic bacteria.[1-4] Diagnosis of bacterial infections and efficient treatment of infectious disease are crucial for human health. The hypothesis that bacteria would likely attain resistance to any antibiotic treatment given the right circumstance and continued emergence of single and multiple antibiotic resistant bacterial strains led to the research focused on antibiotic resistance and elucidation of the mechanisms by which microbes can physically alter a drug's structure, disrupt the interaction between a drug and its cellular target, or alter the behavior and efficiency of its own transport machinery to reduce access to a drug's cellular target.[1,2] To understand the relation between antibiotic induced oxidative stress or impact of oxidized conditions on antibiotic mediated antimicrobial resistance mechanisms, Penicillin was introduced into the structure models of stress proteins UspC (E.*coli*) and USP (Corynebacteriales bacterium X1036) by following the same docking procedure as in the case of dioxygen.[10-13] The estimated free energy of binding and estimated Inhibition constant Ki value for both UspC and USP are -6.45 kcal/mol, 18.70 uM and -1.43 kcal/mol, 89.09 mM respectively.



(a)



(b)

Figure.3. (a) UspC with Penicillin (b) USP with Penicillin

The structure models for UspC and USP incorporated with Penicillin suggested that some amino acid residues are involved in polar, hydrophobic and other types of interactions with nitrogen, carbon and oxygen atoms of β-lactum ring containing antibiotic Penicillin. (Fig.3) In UspC, residues Ser41 and His113 are forming hydrogen bonds with oxygen and nitrogen atoms of Penicillin respectively. Other residues that are involved in hydrophobic interactions with the carbon atoms of Penicillin ring include Ala11, Val12, Leu90, Phe115 and Phe116. Other types of interactions include Glu89 interacting with the carbon atom, Leu90 interacting with nitrogen and hydrogen atoms, Ala11 with the sulfur atom, Ser114 interacting with the carbon atom and Phe115 is interacting with the oxygen atom of the lactum ring of Penicillin. (Fig.3 (a)) In case of USP, the residue Gly119 is forming a hydrogen bond with the nitrogen atom of Penicillin; however, Arg144 is involved in a polar interaction with oxygen atom. (Fig.3 (b))

Discussion:

The antibiotic drug-target interactions and their respective direct effects are well known as discussed above, the bacterial responses to antibiotic drug treatment that contribute to cell death are complex and not as well understood. It is reported that these classes of antimicrobial drugs, regardless of drug-target interactions, all utilize a common mechanism of inactivation whereby they stimulate the production of lethal doses of hydroxyl radicals. The study underlines the effect of oxygen and lactum antibiotic Penicillin on the stress protein derived from both gram + and – bacteria through identified amino acid residues and substrate interactions. Further, antibiotic induced metabolic alterations are associated with oxidative damage to critical cellular components as well as the activation of antioxidant responses. The results suggest that bactericidal antibiotics induce a complex set of metabolic changes in bacteria, downstream of their direct target interaction that correlate with the production of reactive oxygen species (ROS) that can damage key cellular components, oxidize membrane lipids, initiate lipid peroxidation, oxidize proteins and cause DNA damage. Ultimately, since, antimicrobial lethality is typically dependent on hydroxyl radical production/ oxidative stress, which can be countered by bacterial antioxidant responses, targeting such responses may be generally useful in promoting antimicrobial efficacy. Still, a better understanding of the link between stress and antimicrobial resistance, including the identification of the stress induced effectors that promote resistance and/or recruit resistance determinants and the genes involved, is needed to understand the importance of stress responses as resistance determinants, appreciate their value as therapeutic targets and know how best to target them.

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