

Efflux Pumps in Multidrug Resistant Bacteria

An investigation into pump action using molecular modeling

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Introduction

The problem of multidrug resistant bacteria has sparked interest in the mechanism through which these bacteria are resistant to various toxic substances. Gram-negative bacteria have made use of efflux pumps to expel antibiotics from the cell. In *E. coli*, the AcrA-AcrB-TolC efflux pump is responsible for the export of various drugs and toxic substances, including bile salts and short-chain fatty acids [1]. Many of these substrates are lipophilic and amphiphilic. The efflux pump which exports drugs comprises three parts: AcrA, AcrB, and TolC. Different homologues of these components exist in other bacteria and in *E. coli*, but this is the pump responsible for the transport of small molecules.

TolC is a trimer that spans the outer membrane and part of the periplasmic region [2]. Each protomer is made up of 4 α -helices that twist together to form a barrel. This barrel is open to the environment outside of the cell, but at the periplasmic entrance it is closed in a coiled-coil type structure. At this entrance there are six aspartate residues making it extremely electronegative, and this charged area is speculated to be important in substrate movement.

AcrB is a resistance-nodulation-division-type (RND-type) protein that binds a substrate and then uses proton motive force to move it across a membrane. Asp 407, Asp 408, and Lys 940 are three important amino acids that have been identified for the proton motive force powered translocation pathway [3]. AcrB is a trimeric protein made up of a total of 36 α -helices. It is located on the inner membrane and binds to TolC as shown by cross-linking experiments. The asymmetry in the coiled helix structure matches to that of TolC, further suggesting that the two are directly connected. A hydrophobic binding pocket in AcrB, lined by several Phenylalanine residues, binds to many different substrates [4]. Only one protomer is used to bind a substrate at a time [5].

While TolC and AcrB are shown to be connected through cross-linking experiments, their interaction is not energetically favored. To be stabilized, AcrA is needed [6]. AcrA is a membrane fusion protein that is located in the periplasmic region. It is also trimeric and binds to TolC and AcrB to further stabilize the complex. It is speculated that it provides the energy to open the helices of TolC, thus allowing access to the channel. There is some controversy as to whether it also aids in binding the substrate and thus, to some degree, determines substrate specificity [6].

The efflux pump is fully assembled at all times regardless of substrate binding, unlike similar pumps used in protein export, which dissociate regularly [7]. The drug efflux pump uses peristalsis-like movements to expel drugs from the cell. AcrB has a three-step binding change mechanism which is the basis of the peristaltic expulsion of small molecules into the TolC channel [5]. As observed in crystal structures, AcrB exists in three different conformations: *the access state*, where the hydrophobic binding pocket is shrunken but open to the periplasm via a vestibule; *the binding state*, where the vestibule remains open and the binding pocket widens considerably on contact with substrate, while the exit passage into the TolC channel remains blocked by a helix from a neighboring protomer in its extrusion state; and *the extrusion state*, where the movement of a helix (from the neighboring protomer, which was previously

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in the extrusion state) opens up the channel entrance into TolC, while the vestibule closes and the binding pocket contracts, releasing the substrate for expulsion. Now, a helix is extended from this protomer towards the neighboring one in its binding state in order to block its access to the TolC channel.

Although crystal structures provide a strong indication to exactly how efflux pumps work, many questions still arise; some of them being –

1. How is promiscuous substrate binding and expulsion facilitated?
2. Is it possible to deduce any trends of preference shown by the efflux pumps in expulsion of small molecules?
3. What are the different thermodynamic forces that play a role in the movement of molecules through this efflux pump?

A molecular modeling approach can help us address these questions.

Methods

A model for the complete tripartite structure of an efflux pump has recently been published [4]. Present aims include an evaluation of the proposed model, making corrections if required/warranted and finally studying the model by using tools like surface maps (hydrophobicity, electrostatics and Hydrogen bond donor/acceptor distribution maps), docking and scoring [8] using the HINT paradigm [9].

Surface maps have been in existence for several years now [10]. Some very useful property maps include the Ghose-Crippen [11] hydrophobicity maps and the HINT maps [12]. These technologies allow the user to visualize the chemical environment on small molecules or even macromolecules. Maps of this sort shall allow us to identify any conspicuous patterns of hydrophobicity or hydrophilicity which might have a direct repercussion on the passage of small molecules through it during expulsion. An attempt shall be made to connect the hydrophobic maps with the passage of small molecules such as bile salts.

An attempt shall be made to try and develop a model capable of predicting the ability of a pump to increase the minimum inhibitory concentration of any given antibiotic. Molecular properties may be calculated in a spreadsheet and correlated with their tendencies towards expulsion by efflux pumps by statistical analysis (i.e. by multiple linear regression, partial least squares, neural networks, etc.) This should give us a mathematical model which can predict pump activity.

Another approach towards studying efflux pumps shall be to perform a thermodynamic evaluation of pump action. This process would entail a detailed evaluation of the crystal structure of AcrB, along with docking of different substrates into the hydrophobic binding pocket and scoring by HINT. We have worked out a detailed procedure (based on Hess' Law) for theoretical prediction of the free energy of efflux pump action. This procedure shall have to be validated by comparing its predictions with experimentally determined values describing pump action that have been published previously [13]. Our

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aim here is to use simple, well established laws of thermodynamics to predict the feasibility of expulsion for any given molecule.

Possible Conclusions

In previous sections, a preliminary exploratory study of the AcrA-AcrB-TolC efflux pump has been proposed. The major goal of this study is to gain valuable information on the pump's mode of action as well as its preferences in terms of substrates.

One outcome of this research shall be the validation of an efflux pump model that has been proposed previously. This shall establish a basis for several studies in the future including molecular dynamics based investigations into the thermodynamics of pump action, virtual screening for ligands and structure-based drug design.

Another possible outcome shall be to provide a model for prediction of likelihood that a given molecule shall serve as a substrate for this pump.

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