Journal of
Membrane Science & Technology

Chen, J Memb Sci Technol 2012, 2:3
DOI: 10.4172/2155-9589.1000e105

Editorial Open Access

Substrate Specificity and Kinetics of Bacterial Transmembrane Transporters

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Bacterial cell membrane plays a very important role both in the normal physiological functions of the organism and its survival under adverse conditions. Membrane proteins, in particular, are the key players in bacterial membrane functionality. Porins are responsible for the intake of substances such as nutrients and ions for normal activities, whereas other protein complexes, such as efflux pumps, are involved in extruding substances from periplasma to the outside environment. There are many different types of efflux pumps. The Resistance Nodulation Division (RND) of efflux pumps transport a variety of substances including antibiotics, dye, detergents and host derived molecules. Active efflux by the RND system plays an important role in the innate resistance to many different classes of antimicrobial as well as the pathogenesis of some Gram-negative bacteria. The role of RND systems in innate and acquired multiple drug resistance and virulence makes these proteins an attractive target for future development of new generation antibacterial compounds. The representative RND system in major bacterial pathogens are AcrAB-TolC, MexAB-OprM, CmeABC in *Escherichia coli, P. aeruginosa* and *Campylobacter*, respectively. The understanding of the substrate specificity and kinetics of interaction with different substrates amongst these proteins is the prerequisite for the development of efficient antimicrobial strategies.

Traditionally, the best way to characterize the substrate specificity of efflux pumps is to knockout the efflux pump functions in bacteria such as *E. coli* or *Salmonella* and then determine the minimal inhibitory concentrations (MICs) of different substances including antibiotics and detergents. The change of MICs from parental strain to efflux knockout strain is indicative of the substrate specificity of the efflux pump. Since MICs of different compounds result from the combinatorial effects of many different processes including compound intake, efflux pump activity and transportation and action on the targets, the knockout approach to determine the substrate specificity of an efflux pump is found to be misleading in many cases. In addition, this approach is unable to provide information on the kinetic parameters of efflux pump on different substrates. Current studies demonstrated that the MIC and efflux kinetics could be significantly different between different substrates. For example, the efflux kinetics of cloxacillin in *E. coli* was very similar to ampicillin; however, the MIC of cloxacillin in *E. coli* decreased by 512-fold in its *acrB* deletion mutant, whereas the MIC of ampicillin decreased only by 2-fold in such mutant. Analysis of this phenomenon showed that the extensive decrease in MIC in the *acrB* mutant is primarily due to the low permeation of the drug, suggesting that comparison of the MICs between the parental and the *acrB* deletion strains is a very poor measure of the ability of AcrB to extrude antimicrobials [1].

Special approaches are required to characterize the substrate specificity and kinetic parameters of efflux pumps. The crystal structure of the major RND of *E. coli*, AcrAB-TolC has been resolved and found to depict a tripartite complex spanning two membranes, comprising each of the three copies of AcrA, a periplasmic adaptor protein, AcrB, the efflux pump, and TolC, an outer membrane channel. Therefore the best way to determine the kinetic parameter of the AcrAB-TolC system is to maintain the native tripartite structure of the efflux pump. Using intact *E. coli* cells together with a periplasmic β-lactamase, the concentration of β-lactams in the periplasm, a location where most of the substrates are thought to be captured, could be measured. In this model, the velocity of efflux is expressed as the difference between velocity of influx and hydrolysis. Using an optimized *E. coli* model with higher influx rate and higher efflux activity, the kinetic parameters were determined for many β-lactams and the positive cooperativity in the ligand–pump interaction [2]. This approach is only developed for specific antibiotics such as cephalosporins and has not been extended to testing other substrates. The recent advanced understanding on the structure of AcrAB-TolC has opened up some new approaches to characterize the kinetic of this efflux pump. It was shown that two discrete multisite binding pockets of AcrB bind to high and low molecular mass drugs, respectively [3]. The twisting of the AcrB efflux pump will enable the extrusion of substrate to the TolC channel. Therefore the binding affinity of substrates to AcrB indirectly reflects the substrate specificity and kinetics of the efflux pumps. Based on the computational biological tool such as molecular docking, it is possible to predict the binding of different substrates to efflux pump. Further characterization and confirmation by Isothermal Titration Calorimetry (ITC) will enable us to acquire the K_d of different substrate to the efflux. This approach can be considered as a preliminary screening method to determine the substrate specificity and kinetic constants of an efflux pump.

In conclusion, the knowledge of substrate specificity and kinetic constants is essential in our attempts to understand the contribution of the pumps to drug resistance in a quantitative manner and the development of new antibacterial strategies. Nevertheless, it has been very difficult to obtain kinetic constants for an efflux pump. The combinatorial uses of different approaches may aid us in gaining better understanding of the kinetics of different bacterial membrane transporters.

References

1. Lim SP, Nikaido H (2010) Kinetic parameters of efflux of penicillins by the multidrug efflux transporter AcrAB-TolC of *Escherichia coli*. Antimicrob Agents Chemother 54: 1800-1806.
2. Nagano K, Nikaido H (2009) Kinetic behavior of the major multidrug efflux pump AcrB of *Escherichia coli*. Proc Natl Acad Sci USA 106: 5854-5858.
3. Nakashima R, Sakurai K, Yamasaki S, Nishino K, Yamaguchi A (2011) Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket. Nature 480: 565-569.

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Received July 11, 2012; **Accepted** July 11, 2012; **Published** July 13, 2012

Citation: Chen S (2012) Substrate Specificity and Kinetics of Bacterial Transmembrane Transporters. J Memb Sci Technol 2:e105. doi:10.4172/2155-9589.1000e105