Structure and Function of the Drug Efflux Transporter

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The emergence of bacterial multidrug resistance is an increasing problem in the treatment of infectious diseases. The major cause for the multidrug resistance of bacteria is a multidrug efflux transporter, which exports drugs out of the cells. AcrB and its homologues are the major multidrug efflux transporter in gram-negative bacteria, which confer intrinsic drug tolerance and multidrug resistance when they are overproduced. AcrB exports a wide variety of antibiotics, antiseptics, anti-cancer chemotherapeutics and toxic compounds including anionic, cationic, zwitterionic, and neutral compounds directly out of the cells driven by proton-motive force. In order to understand molecular mechanism of multidrug recognition and active transport by the multidrug transporter, we performed X-ray crystallographic analysis of this transporter. In 2002, the first crystal structure of AcrB was solved by our group [1]. And in 2006, we solve the complex structures of AcrB with substrates in the new crystal form [2]. The crystals used in the study have lower crystallographic symmetry than that in previous crystals. In the new crystal form, AcrB-drug complex consists of asymmetric three protomers, each of which has different conformation corresponding to one of the three functional states of the transport cycle. They are “access” in which substrate is incorporated, “binding” in which substrate is binding, and “extrusion” in which substrate has just extruded. Bound substrate was found in the periplasmic domain of “binding” protomer. In the periplasmic part of each protomer, there is a substrate-binding pocket. In this pocket, there are many aromatic amino acid residues for binding of hydrophobic substrates by aromatic- aromatic interactions. Different side chains in the pocket form different binding sites to recognize different type of substrates. The mechanism, the multi-site binding, for multiple substrate recognition was also found in the soluble multidrug binding transcription factor [3]. Substrates are incorporated from opened vestibule in the “access” state. Then, they bind to the different locations in the voluminous aromatic pocket in the “binding” state. Finally, in the “extrusion” state, the bound substrates are extruding to the connecting TolC channel by shrinking the pocket.

Based on three different conformations and transport states observed in the three protomers of AcrB, we propose that drugs are exported by a three-step functionally rotating mechanism in which drugs undergo ordered binding change [2]. Such an ordered binding change mechanism in trimeric protein complex is very similar in principal to the mechanism of the pseudo-trimeric F1ATPase. But in AcrB, there is no central stalk (subunit) that undergoes mechanical rotation.

In the crystal structure solved in 2002, trimeric AcrB has symmetric structure by the crystallographic three-fold symmetry. All three protomers have the structure comparatively similar to the “access” state in the new crystal structure. In many cases, a homo-origomeric protein complex purposely breaks its symmetry to facilitate their function. Allostery and cooperative controls are common examples and smart strategies of the protein function through the asymmetric property. Structural fluctuations between these conformers are generally very small and sometimes these small structural differences might be constrained by the external crystallographic packing force.

[1] Murakami, S. *et al.,* ***Nature,*** **419,** 587 (2002) [2] Murakami, S. *et al.,* ***Nature,*** **443,** 173 (2006)

[3] Schumacher, M.A. *et al.,* ***Science,*** **294,** 2158 (2001)