Crystal Structures of a Bacterial Multidrug Transporter Reveal a Functionally Rotating Mechanism

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, anticancer chemotherapeutics and toxic compounds including anionic, cationic, zwitterionic, and neutral compounds directly out of the cells; these compounds bypass the periplasm and are driven by proton-motive force. AcrB cooperates with a membrane fusion protein AcrA and an outer membrane channel TolC. The X-ray crystal structure of AcrB was first elucidated by our group in 2002 [1]. It is the first structure of not only a multidrug efflux transporter but also a secondary active transporter, driven by proton-motive force [2].

Now, we elucidated the crystal structures of AcrB with and without substrates in the new crystal form [3]. The crystal used in this study has a lower crystallographic symmetry than the crystal used in our previous study. The new crystal structure solved with the new crystal form is asymmetric (Fig. 1). The AcrB-drug complex consists of three asymmetric protomers, each of which has a different conformation corresponding to one of the three functional states of the transport cycle. The three monomers are named as "access" in which the substrate is incorporated (green), "binding" to which the substrate is bound (blue), and "extrusion" by which the substrate is extruded (red).

The bound substrate was found in the periplasmic domain of the "binding" protomer (Fig. 1). In the periplasmic part of each protomer, there is a substrate-binding pocket. In this pocket, there are many aromatic amino acid residues for the binding of hydrophobic substrates by aromatic-aromatic interactions. Different side chains in this pocket form different binding sites to recognize different types of substrate. The mechanism, the multisite binding, for multiple substrate recognition was also found in the soluble multidrug binding transcription factor. The pocket is expanded in the "binding" state and allows substrate binding in this voluminous pocket. The expansion of the binding pocket creates a possible exit between subdomains forming the pocket. However, one functionally important α-helix belonging to the "extrusion" protomer inclines and fills this exit to make the pocket closed (Fig. 1(b): dotted circle) [4].

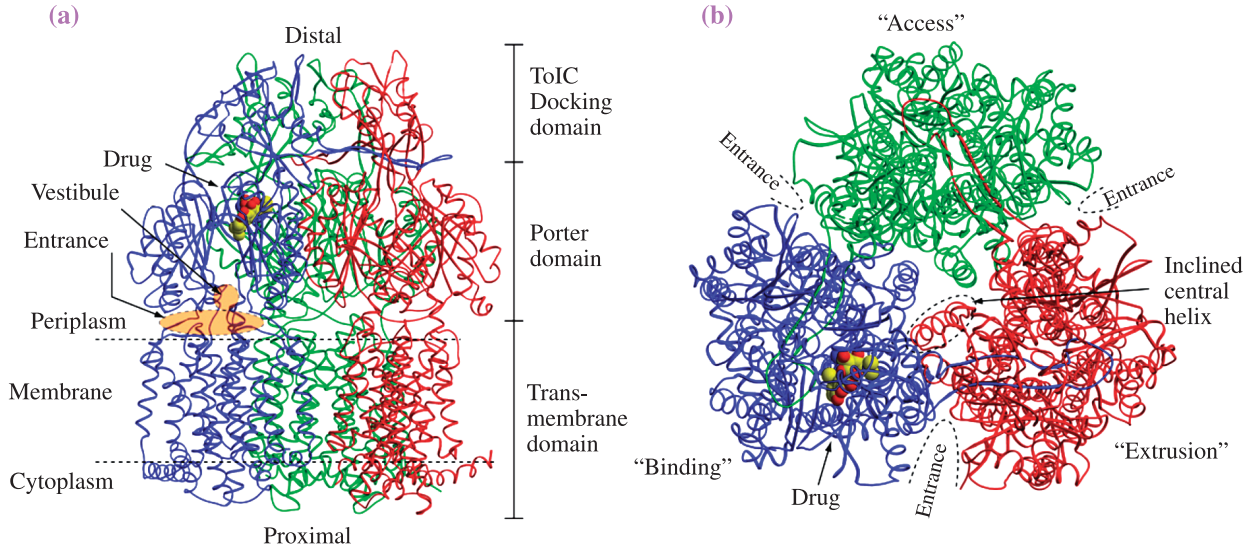

Fig. 1. Structure of the AcrB-drug (minocycline) complex.
Three protomers are individually colored. **(a)**: Ribbon representation viewed from the side parallel to the cytoplasmic membrane plane. The minocycline molecule is shown in CPK representation. The extra-membrane (periplasmic) headpiece is at the top and the transmembrane region is at the bottom. **(b)**: Top view from distal side of the cell.

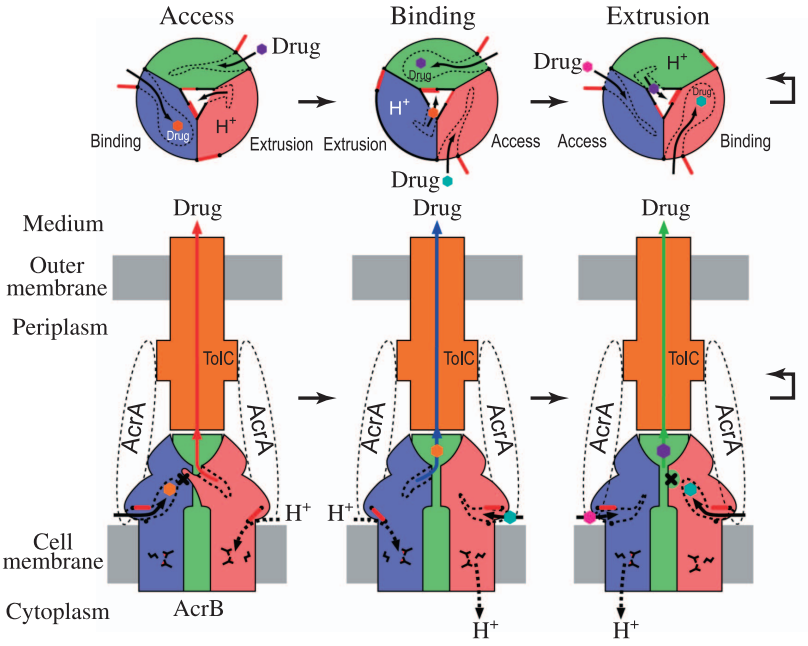

Fig. 2. Schematic illustration of the proposed "functionally rotating ordered multidrug binding change mechanism" mediated by AcrB.
Colors and arrangements in the left panel correspond to those in Fig. 1. **(a)**. The top view from the distal side of the cell. The view from the side parallel to the membrane plane. The entrance and exit within each protomer are depicted as red flaps. The drug binding pocket and translocation pathway are represented as a dotted line. Drugs are illustrated as hexagons.

At the same time, this inclination creates an open space from the shrunk binding pocket in the "extrusion" protomer. Thus, this situation corresponds to just after the extrusion of substrates from the pocket to the exit of the AcrB, which connects to the outer membrane channel, TolC [5]. The remaining protomer "access" also has a shrunken binding pocket, but an open "vestibule" exposed to the periplasmic space. Substrates are incorporated from open vestibule in the "access" state, and bind to the different locations in the voluminous aromatic pocket in the "binding" state. Then, in the "extrusion" state, the vestibule is closed and the exit is opened. At this state, the bound substrate is squeezed out into the TolC channel by shrinking the pocket. All these structural changes are coupled to proton translocation across the membrane (Fig. 2). The protonation in the "extrusion" state and deprotonation in the "access" state of three functionally important charged residues (Asp407, Asp408 and Lys940) in the transmembrane domains would affect the accessibility or influence binding or extrusion of substrate that occurring in the periplasmic domains.

On the basis of the three different conformations and transport states observed for 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 (Fig. 2) [3]. Such an ordered binding change mechanism in a trimer is similar in principle to the mechanism of the trimeric F1ATPase, except that AcrB has no central stalk that undergoes mechanical rotation.

The experiments were performed at beamlines **BL41XU** and **BL44XU**.

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References

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