Peristaltic mechanism of bacterial multidrug efflux transport

Multidrug resistance of pathogenic bacteria is a serious problem in current chemotherapy. Multidrug efflux transporters are the major cause of the multidrug resistance of Gram-negative bacteria. Unfortunately, there is no clinically useful drug against multidrug efflux transporters. We first determined the crystal structure of a major multidrug efflux transporter, AcrB, in *E. coli* [1] and then revealed its drug binding structures [2,3].

The most significant characteristic of multidrug efflux transporters is their extraordinarily wide range of substrates including both aromatic and aliphatic compounds. It is difficult to determine the common chemical structures of their substrates. However, multidrug transporters actually distinguish between substrates and others. Our first drug-free structure determination of AcrB in 2002 [1] revealed that it is a homotrimer that acts as a membrane vacuum cleaner, taking up its substrates from the outer surface of the cell membrane and extruding them outside the outer membrane through the outer membrane channel TolC. Since drugs and toxic compounds generally invade cells through the lipid matrix of the cell membrane, this mechanism efficiently stops their invasion at the border. Then, we determined the minocycline- and doxorubicin-binding structures of AcrB in 2006 [2]. Only one drug molecule bound to the AcrB homotrimer. Three monomers show different conformations corresponding to the intermediate steps of drug transport, that is, *access*, *binding*, and *extrusion*. A drug molecule binds only to the *binding* monomer. The *access* and *binding* monomers are inside-open while the *extrusion* monomer is outside-open. Drugs are exported vie ordered conformational change of these three conformations. This mechanism was named the functionally rotating mechanism. When bound minocycline and doxorubicin molecules are superimposed, they only partially overlap. Large parts of both molecules interact with different sets of amino acid residues in their binding site; this is the so-called “multisite-drug binding”, which is a structural basis of multidrug recognition.

As for energy coupling, the driving force of drug efflux is a proton motive force. In the transmembrane region, there are three acidic and basic residues, Asp407, 408 and Lys940, at the middle of TM4 and TM10. In *access* and *binding* monomers, these residues form ion pairs, but in the *extrusion* monomer, the ion pair is dissociated, followed by the two Asp (TM4) and one Lys (TM10) being about 45° twisted in each other probably due to protonation/deprotonation during proton translocation. This conformation change is transmitted to the periplasmic domain to cause a series of conformational changes from *binding* to *extrusion* stages. Therefore, drugs are actively exported by a remote-conformational change.

In 2011, we determined the new drug-binding structures with the high-molecular-weight drugs, rifampicin and doxorubicin (Fig. 1) [3]. Similar to minocycline and doxorubicin, one molecule binds to AcrB trimer, however, surprisingly, the bound monomer is not a *binding* monomer but an *access* monomer. We found an additional multisite drug-binding pocket, named the proximal pocket, active at the *access* stage (Fig. 2). The proximal pocket and the former distal pocket are arranged on the drug translocation channel and separated by a Phe617 loop, which swings, during conformation change from *access* to *binding* stages

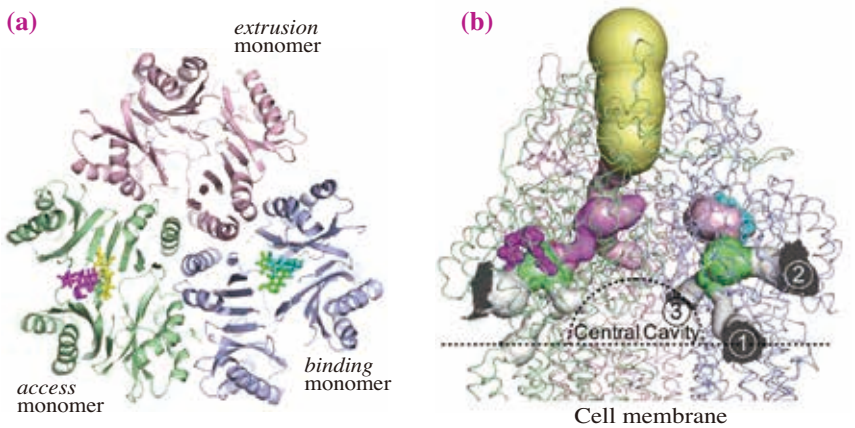

Fig. 1. Drug-binding AcrB structures. **(a)** Horizontal cutaway view of the porter region of AcrB with superimposed bound drugs, rifampicin (purple), erythromycin (yellow), minocycline (blue) and doxorubicin (green). **(b)** Side view of the periplasmic domain of AcrB trimer with intramolecular channels and bound drugs. Intramolecular channels including proximal (green) and distal (pink) binding pockets and the exit funnel (yellow) are depicted by the program CAVER.

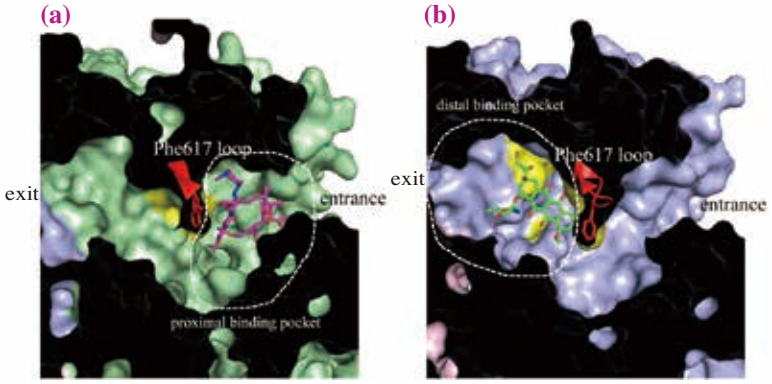

Fig. 2. Cutaway view of the intramolecular channels of the porter region of AcrB. **(a)** Access monomer with bound rifampicin (purple). **(b)** Binding monomer with bound doxorubicin (green). Phe617 loop is depicted in red.

and contributes to the pumping of drugs from proximal to distal pockets. The substrate specificities of both pockets would be different from each other. High-molecular-weight drugs tightly bind to the proximal pocket, and then they are pumped to the distal pocket by protein conformational change and irreversibly occluded in the distal pocket. On the other hand, low-molecular-weight drugs would enter into and tightly bind to the distal pocket without tight binding to the proximal pocket. Therefore, the bindings of high- and low-molecular-weight drugs to the AcrB trimer do not compete with each other. We actually obtained an AcrB crystal in which minocycline and rifampicin simultaneously bind to its distal and proximal pockets, respectively. However, the efflux of distal-binding drugs is competitively inhibited by proximal-binding drugs, indicating that distal- and proximal-binding drugs use the same drug translocation pathway.

In order to verify the role of both pockets in drug transport, we constructed several mutants in proximal and distal pockets. Mutations of proximal residues interacting with proximal-binding drugs showed complete loss of erythromycin (proximal-binding drug) efflux but unaffected doxorubicin (distal-binding drug) efflux. In those mutants, erythromycin no longer inhibits doxorubicin efflux. On the other hand, mutations in distal pocket caused a great decrease in doxorubicin efflux, but only showed a limited degree of effect on erythromycin efflux, indicating that specific interaction with residues in the distal pocket is not necessary for proximal-binding drug transport. The results in this paper revealed that drugs are pumped through the intramolecular translocation channel of AcrB via the entrance → proximal pocket (*access* stage) → distal pocket (*binding* stage) → exit (*extrusion* stage) by a peristaltic motion of the protein (Fig. 3). The presence of dual multidrug binding pockets with different binding spectra would contribute to expanding the substrate specificity. In addition, it should be noted that there are three different entrances that are merged at the proximal pocket (Fig. 1(b)). Three entrances open to the surface of the cell membrane (entrance 1), periplasm (entrance 2) and the central cavity (entrance 3), respectively. Among them, entrances 1 and 2 are proved to act *in vivo* during drug efflux by mutagenesis studies. Such multi-entrances would also contribute to expanding the range of substrates.

In conclusion, our structure determination revealed that the bacterial multidrug efflux transporter is a peristaltic drug efflux pump having dual multisite pockets and multi-entrances. This is the structural basis of this extremely high-performance multidrug efflux pump. These findings should facilitate the structure-based development of efflux-transporter inhibitors.

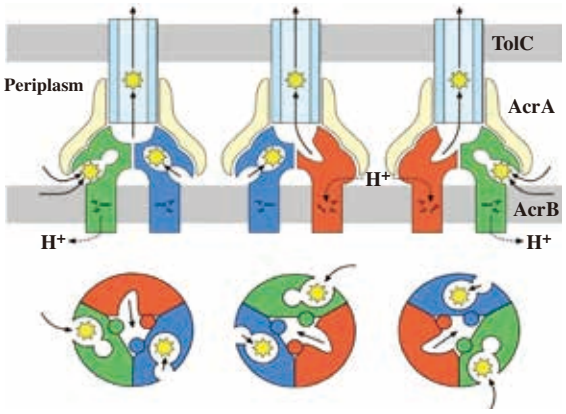

Fig. 3. Peristaltic mechanism of drug transport mediated by AcrB. Drugs first enter into the proxymal pocket at the access stage, translocate to the distal pocket at the binding stage, and finally, are released from the exit at the extrusion stage. Green, blue and red colors indicate the access, binding and extrusion stages, respectively.

Ryosuke Nakashima and Akihito Yamaguchi*

Department of Cell Membrane Biology,
Osaka University

*Email: akihito@sanken.osaka-u.ac.jp

References

[1] S. Murakami *et al.*: Nature **419** (2002) 587.
[2] S. Murakami *et al.*: Nature **443** (2006) 173.
[3] *R. Nakashima, K. Sakurai, S. Yamasaki, K. Nishino and A. Yamaguchi: Nature* ***480*** *(2011) 565.*