Crystal Structure and Function of MexAB-OprM Antibiotic Efflux Pump Subunits of *Pseudomonas aeruginosa*

Emergence of bacteria resistance to many different antibiotics and of drug-resistant cancer cells is a great concern in human health. An important factor contributing to the multidrug resistance would be the drug efflux pump, which lowers the intracellular drug concentration by exporting incoming chemotherapeutic agents across the membranes. *Pseudomonas aeruginosa* often infects hospitalized patients whose immune activity is lowered by some reasons, and such an infection is often life-threatening. The multi-antibiotic resistance of this organism is largely attributable to the production of multidrug efflux pumps.

The MexAB-OprM efflux pump of *P. aeruginosa* consists of three membrane-bound subunits, MexA, MexB and OprM, anchored to the inner and outer membranes, respectively. The MexB subunit crosses the cytoplasmic membrane 12 times, selects and transports the antibiotics [1]. The crystal structure of an MexB homologue, AcrB of *Escherichia coli*, showed that the protein consists mainly of three domains: the membrane-spanning domain, the pore domain and the TolC-docking domain [2]. The OprM subunit is the outer membrane-anchored lipoprotein that is assumed to form the antibiotic discharge duct across the outer membrane. The MexA subunit anchors to an inner membrane via N-terminal fatty acids [3]. Removal of fatty acids liberates a protein from the membrane and the protein becomes freely soluble in aqueous solutions. Therefore, MexA is assumed to link MexB and OprM, helping the assembly of the functional pump unit. For better understanding of the role of the antibiotic efflux pump subunits, we analyzed the X-ray crystal structures of OprM and MexA (**BL44XU**).

[OprM structure] We studied the X-ray crystallographic structure of OprM at 2.56 Å resolution [4]. The overall structure exhibited a trimeric assembly of the OprM monomer that consisted mainly of two domains: the membrane-anchoring β-barrel and the cavity-forming α-barrel (Fig. 1). OprM anchored to the outer membrane via two modes of membrane insertions. One was via covalently attached N-terminal fatty acids and the another via the β-barrel structure consensus on intrinsic outer membrane proteins. The β-barrel had a pore with a diameter of about 6 to 8 Å, which is not sufficiently large to accommodate the exit of any antibiotics (Fig. 1). The α-barrel was about 100 Å long formed mainly by a bundle of α-helices that formed a solvent-filled cavity of about 25,000 $Å^3$. The proximal end of the cavity was tightly sealed, thereby not permitting the entry of any molecule. This structure showed that the resting state of OprM had a small outer membrane pore and a

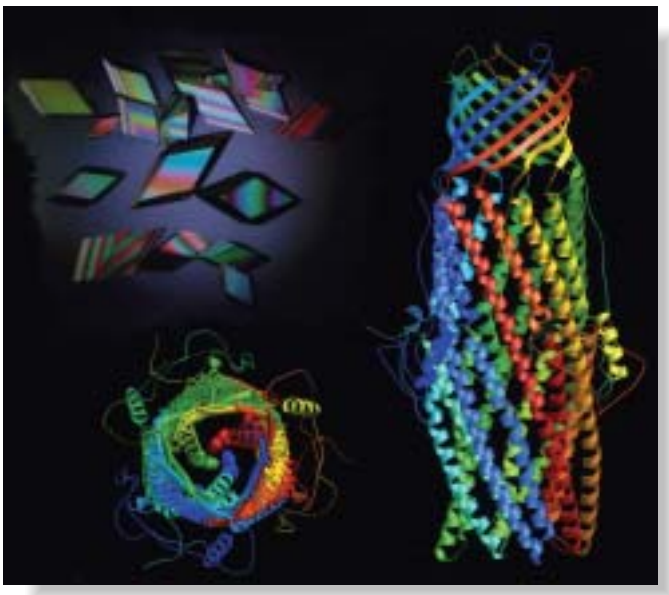

Fig. 1. Structure of OprM. Right figure illustrates a side view of the OprM trimer. The monomer is colored in a gradient from yellow to red. Left-lower image illustrates the top view of the trimer featuring an open top and a tightly sealed bottom.

tightly closed periplasmic end, which sounds plausible because the protein should not allow free access of antibiotics.

[MexA structure] We showed the X-ray crystallographic structure of MexA at a resolution of 2.40 Å [5]. The global MexA structure showed unexpected new features with a spiral assembly of six and seven monomers that were joined together at one end by a pseudo two-fold image (Fig. 2). The monomer showed a new protein structure with a tandem arrangement consisting of at least three domains and presumably one more. (i) The rod domain had a long hairpin of twisted coiled-coil that extended to one end. (ii) The second domain adjacent to the rod α-helical domain was globular and constructed by a cluster of eight short β-sheets. (iii) The third domain located distal to the α-helical rod was globular and composed of seven short β-sheets and one short α-helix. The structure suggests that MexA functions as a molecular clump connecting MexB and OprM.

On the basis of these results, we constructed a model of the MexAB-OprM pump assembly (Fig. 3). The pump assembly cross bridge the inner and outer membranes. The substrates taken up by MexB may be injected into the OprM cavity by the energy of proton motive force across the inner membrane. MexA clumps the MexB and OprM. OprM forms the antibiotic-ejecting duct.

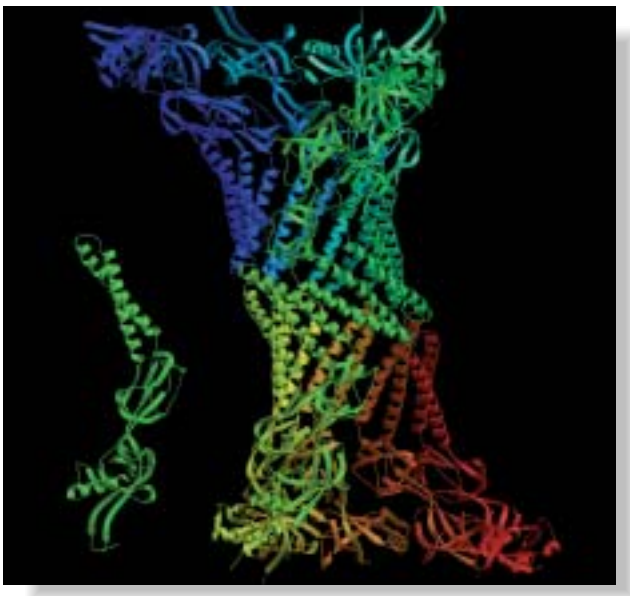

Fig. 2. Crystal structure of MexA. Right figure illustrates a tridecamer of MexA and left figure is a monomeric form.

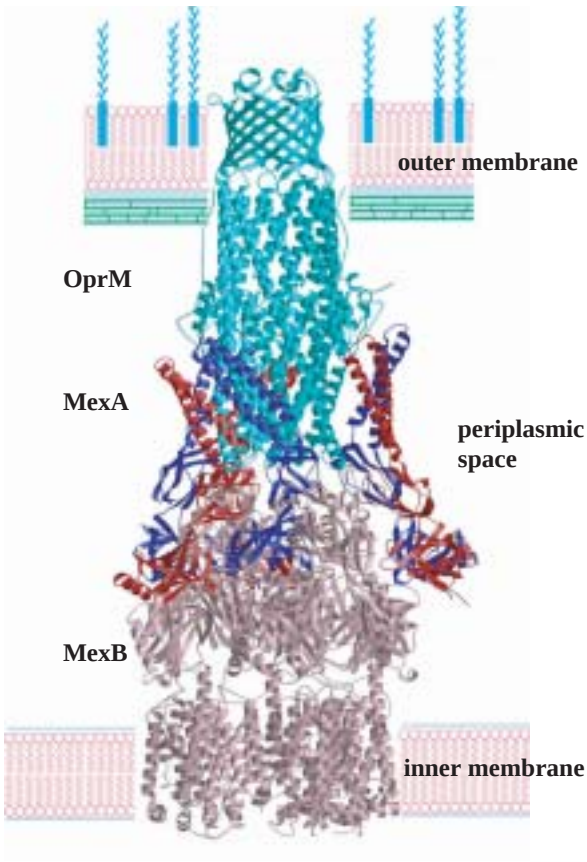

Fig. 3. Proposed assembly model of MexAB-OprM antibiotic efflux pump.

Taiji Nakae [a,b]

(a) Tokai University School of Medicine
(b) The Kitasato Institute

E-mail: nakae@is.icc.u-tokai.ac.jp

References

[1] S. Eda *et al.*: J. Biol. Chem. **278** (2003) 2085.
[2] S. Murakami *et al.*: Nature **419** (2002) 587.
[3] H. Yoneyama *et al.*: J. Biol. Chem. **275** (2000) 4628.
[4] *H. Akama, M. Kanemaki, M. Yoshimura, T. Tsukihara, T. Kashiwagi, H. Yoneyama, S. Narita, A. Nakagawa and T. Nakae: J. Biol. Chem.* ***279*** *(2004) 52816.*
[5] *H. Akama, T. Matsuura, S. Kashiwagi, H. Yoneyama, T. Tsukihara, A. Nakagawa and T. Nakae: J. Biol. Chem.* ***279*** *(2004) 25939.*