Crystal structure of multidrug transporter MATE

Toxic compounds essentially must be exported from the cell to maintain life. MATE (multidrug and toxic compound extrusion) transporters function in the efflux of endogenous cationic and lipophilic substances using an electrochemical gradient of H^+ or Na^+ across the membrane [1], and are ubiquitously distributed in archaea, bacteria, and eukaryotes. Bacterial MATE transporters confer multidrug resistance (MDR) to pathogens, such as multi-antibiotic-resistant *Staphylococcus aureus*, which has received much attention as a hospital-acquired infection. In cancer cells, MATE transporters export structurally diverse anti-cancer drugs, causing fatal reductions in the therapeutic efficacies of anti-cancer drugs. Thus, the demand for the discovery of inhibitory molecules of MATE transporters is high because of their clinical importance.

PfMATE was crystallized using the lipidic cubic phase (LCP) method. The tiny crystals (20~30 μm) grown in LCP diffracted X-rays to 2.2 Å resolution at maximum on beamline **BL32XU**. Diffraction data sets were collected by the helical data collection method using a microbeam with a 1 μm width and a 5 μm height [2]. Co-crystallization with the cyclic peptides (generated by the RaPID system [3]) improved the quality of the SeMet-derivatized crystals, which facilitated the phase determination by the single-wavelength anomalous diffraction (SAD) method. The peptides may restrict the rocker-switch motion of the lobes and lead to improved quality. Additionally, the cyclic peptides have the ability to inhibit the extrusion function of PfMATE. Finally, we determined the outward-open structures in two different conformations ("straight" and "bent" conformations at 2.4 and 2.5 Å resolutions, respectively) (Fig. 1).

The structure of PfMATE consists of 12 transmembrane-helices (TMs) forming two pseudo-symmetrical lobes, an N-lobe (TM1-TM6) and a C-lobe (TM7-TM12), which are related by a pseudo-two-fold symmetry axis. Therefore, the rocker-switch mechanism similar to that employed by the major facilitator superfamily (MFS) transporters was proposed [4]. The PfMATE structure adopts a V-shaped conformation, with the central cleft open toward the extracellular side, representing an outward-open state. A large hydrophobic central cleft can be divided into two cavities, the N- and C-lobe cavities. The N-lobe cavity is larger than the C-lobe cavity. In the outward-open state, we found that it adopts two distinct conformations, the "bent" and "straight" conformations, in terms of the structure of the TM1 helix in the N-lobe (Fig. 1). In the straight conformation, TM1 forms a single and straight helix, whereas in the bent conformation, it is unstrained and kinked at Pro26 and Gly30 and bent toward the TM2 side. As a result, the N-lobe cavity, which is mainly formed between TM1 and TM2, is collapsed in the bent conformation. To investigate the importance of Pro26, we mutated Pro26 to Ala and Ile, which markedly reduced the transport activities, although the membrane expression of all of the mutants was confirmed. TM1 is kinked at Pro26 in the bent conformation, which is essential to drug export activity.

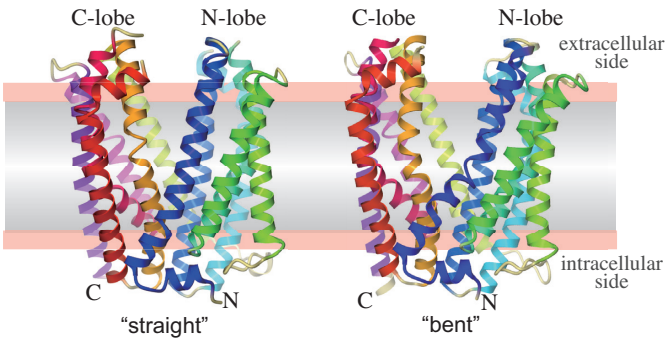

Fig. 1. Overall structures of PfMATE. Schematic representations of the two conformations of PfMATE, viewed from the membrane. The molecules are shown as ribbons with each helix colored as a rainbow, from the N terminus (blue) to the C terminus (purple).

A significant rearrangement of the side chain interactions was also observed between the straight and bent conformations, notably at the apex of the N-lobe cavity (Fig. 2(a)). Some acidic residues are clustered in this site. In the straight conformation, Asp184 is within hydrogen bonding distance with Asp41 (2.8 Å), suggesting that the carboxylate group of either Asp41 or Asp184 is protonated. In view of the fact that Asp184 is buried in the protein core while Asp41 is exposed to the bulk solvent, it is likely that Asp184 is protonated and Asp41 is deprotonated. In contrast, in the bent conformation, Asp41 is closer to TM2. Asp184 also forms a direct hydrogen bond with Asp41. This interaction network is further surrounded by the hydrophobic environment, and sequestered from the solvent region of the extracellular half channel. Therefore, in the bent conformation, the Asp41 and Asp184 side chains are probably both protonated, since a charged group in such a low-dielectric environment inside the protein is energetically unfavorable. This is consistent with the fact that the bent-form crystals appeared under acidic conditions. To investigate the importance of these acidic residues in the N-lobe cavity, we performed complementation assays of Asp41 and Asp184 mutants. These mutations abolished both the drug

and H^+ transport activities, indicating the importance of these acidic residues in the antiport mechanism. The change in the protonation state of the Asp41 side chain may trigger the rearrangement, thereby inducing the structural conversion from the straight conformation to the bent conformation.

To investigate the drug recognition mechanism, we determined the 2.9-Å-resolution crystal structure of PfMATE in the presence of a norfloxacin derivative compound (Br-NRF). In this complex structure, an electron density that resembles the shape of the Br-NRF molecule was observed in the N-lobe cavity (Fig. 2(b)). The Br-NRF molecule is mainly recognized by shape complementarity. To further explore the roles of these residues in substrate recognition, we performed complementation assays of their mutants. The Ala mutants of Met173, Asn180, and Met206 abolished the function, indicating the importance of these residues in the recognition of the substrates.

We further determined the macrocyclic peptide complex structure in two distinctive binding modes. The MaL6 peptide consists of a 17-aa macrocyclic structure. In the complex structure with MaL6, PfMATE adopts the straight conformation, and the cyclic peptide binds between the N- and C-lobes by forming a β-hairpin structure. The peptide mainly interacts with the extracellular side of TMs 2, 7, and 8, but does not interact with the N-lobe cavity. Therefore, the interaction is limited to the extracellular entrance of PfMATE. In contrast, the MaD5, which has a lariat like structure containing a 7-aa macrocycle, deeply bind within the central cleft. Thus, these peptides may provide new scaffolds for the development of potent inhibitors against MATE transporter families from bacteria and eukaryotes.

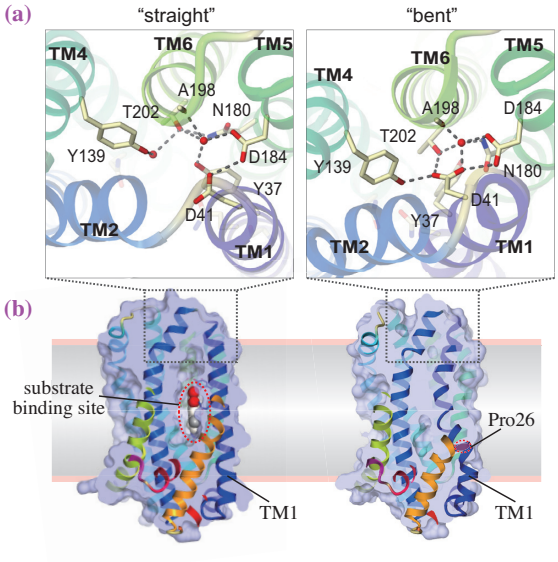

Fig. 2. Differences between two conformations. **(a)** Close-up views of the interaction networks in the two forms at the apex of the N-lobe cavity. Black dashed lines indicate hydrogen bonds. **(b)** Surface models viewed from the central cleft, highlighting the N-lobe cavity. The Br-NRF molecule is depicted by a space-filling model in the N-lobe cavity of the straight conformation.

In this study [5], we proposed the last step of the H^+-driven drug extrusion mechanism by MATE, on the basis of the present structural and functional analyses (Fig. 3). After the structural conversion from the inward-open state to the outward-open state, PfMATE is deprotonated, and the drug substrate resides in the cavity (corresponding to the present Br-NRF complex straight form structure). The protonation of Asp41 induces the bending of TM1 at Pro26, which collapses the N-lobe cavity and extrudes the bound substrate into the extracellular space (corresponding to the present bent form structure). Finally, the protonated transporter undergoes further structural conversion to the inward-open state. The complete understanding of the H^+/drug antiport cycle of MATE awaits the elucidation of the structures capturing the occluded and inward-open states.

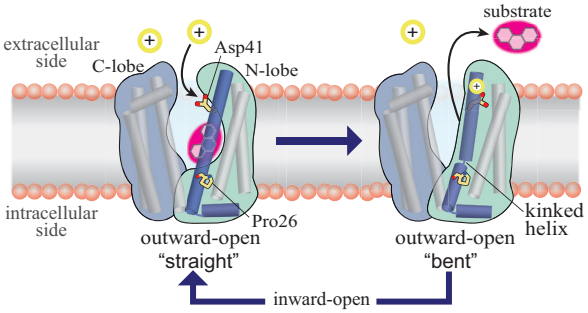

Fig. 3. Drug extrusion mechanism by MATE. In the drug-binding, "straight" outward-open conformation, a cation (yellow) binds to the conserved acidic cluster. Cation binding to D41 induces TM1 (blue) bending and changes the conformation to the "bent" form, which extrudes substrate drugs (magenta). Through the inward-open state, the substrate binding induces structural changes back to the outward-open state.

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References

[1] M.H. Brown *et al.*: Mol. Microbiol. **31** (1999) 394.
[2] K. Hirata *et al.*: AIP Conf. Proc. **1234** (2010) 901.
[3] C.J. Hipolito and H. Suga: Curr. Opin. Chem. Biol. **16** (2012) 196.
[4] Y. Yin *et al.*: Science **312** (2006) 741.
[5] *Y. Tanaka, C.J. Hipolito, A.D. Maturana, K. Ito, T. Kuroda, T. Higuchi, T. Katoh, H.E. Kato, M. Hattori, K. Kumazaki, T. Tsukazaki, R. Ishitani, H. Suga and O. Nureki: Nature* ***496*** *(2013) 247.*