論文の内容の要旨

Structural and functional analyses of eukaryotic MATE transporter (真核生物由来 MATE トランスポーターの構造機能解析)

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Multidrug extrusion is the essential biological process for maintaining the cellular homeostasis. All domains of life have been developed and acquired strategies for elimination of harmful xenobiotics. One of the most important strategy for detoxification of xenobiotics is export them from the cells with membrane proteins called as multidrug transporters. There are six member of multidrug transporters and they work independently to achieve extrusion of xenobiotics. The membrane transporters are divided into two large groups, one is the primary active transporters which transport substrates coupling to ATP hydrolysis, and the other is the secondary active transporters which use chemical gradient across the membrane.

Multidrug And Toxic compounds Extrusion (MATE) transporters belong to the secondary active multidrug transporters. MATE transporters extrude various organic cations using the H^+ or Na^+ chemical gradients, and conduct the electroneutral antiport.

MATE transporters are conserved in all domains of life. In bacteria, they work as efflux pumps of xenobiotics including antibiotics, and they give bacteria multidrug resistance. In human body, MATE transporters are expressed at the kidney and the liver, and they export various clinical drugs, including cimetidine, metformin, procainamide, cephalexin, and acyclovir into urine and bile using H^+ chemical gradient. Then MATE transporters determine the plasma concentration of these drugs, and MATE transporters are the important factor in the pharmacodynamics of clinical drugs. In plant, MATE

transporters are suggested that they have various biological roles in addition to the xenobiotics extrusion, considering that more than 50 MATE paralogues exist in *Arabidopsis thaliana*. The plant MATE transporters mediate the transport process of secondary metabolites, such as nicotine, flavonoids and proanthocyanidin precursors, and plant hormones, including abscisic acid. Furthermore, plant MATE transporter mediate citrate export from the root cells for chelation of phytotoxic aluminum ions, confering tolerance towards harmful aluminum in acidic soils.

Based on the amino-acid similarity, MATE transporters are classified into three subfamilies; NorM-type MATE subfamily, DinF-type MATE subfamily, and eukaryotic MATE subfamily, respectively. All prokaryotic MATE transporters belong to either NorM-type MATE or DinF-type MATE subfamily, on the other hand, all MATE transporters expressed in eukaryotes belong to eukaryotic MATE subfamily.

The crystal structures of NorM-type and DinF-type MATE transporters have been reported, and then the detailed mechanisms of transport and substrate recognition of prokaryotic MATE transporters are being revealed gradually. Although structural bases of NorM-type and DinF-type have revealed, that gave us limited clues for understanding the detailed mechanism of eukaryotic MATE transproters because of their low amino-acid similarity. None of crystal structures of eukaryotic MATE transporters had been reported because of their difficulty in crystallization, so the detailed mechanism remains elusive.

Here we determined the crystal structure of AtDTX14 from *Arabidopsis thaliana* at 2.6 Å resolution as eukaryotic MATE transporter. The structure of AtDTX14 is composed of two lobes; N-lobe (TM1-6), and C-lobe (TM7-12), as is observed in the structures of prokaryotic MATE transporters. The crystal structure of AtDTX14 suggested that the substrate binding pocket is located at the center of C-lobe and the pair of acidic residues plays an important role in substate recognition and H^+ binding. Molecular dynamics simulations and structure guided biochemical analyses proposed the detailed substrate extrusion mechanism, in which the protonation of that acidic residues induces the conformational change of TM7 and this makes the substrate binding pocket shrink. Furthermore, the molecular dynamics with external force proposed the inward-open state of AtDTX14 and the validity of the extracelluar gate is confirmed with the evolutionary coupling pair calculation analysis.

Structural and functional analyses of AtDTX14 gave us insight into the structural basis of transport cycle in eukaryotic MATE transporters.