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Plasticity in the proton interactions of the major facilitator superfamily multidrug transporter LmrP

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Abstract

Microbes have the ability to develop resistance to cytotoxic compounds and drugs, and to adapt themselves to the changes in the exposure to these compounds and thus this is an extremely important medical problem. Resistance to drugs can be either specific for a single drug or class of drugs, or to a diverse range of toxic compounds that are structurally and functionally unrelated. The latter phenomenon is called multidrug resistance, which is an important mechanism of resistance by bacteria to most of the available antibiotics. The occurrence of multidrug resistance majorly is due to the increased expression levels of drug efflux pumps in the cell. These pumps are involved in the extrusion of antimicrobial reagents across the cell membrane, away from intracellular targets causing antimicrobial/ anti-cancer resistance.

The mutants were identified based on a homology model of LmrP based on the crystal structure of the glycerol-phosphate/phosphate antiporter GlpT. Ethidium transport studies were conducted in Lactoccuslactis intact cells. LmrP and mutant proteins were reconstituted in proteoliposomes prepared from washed E. coli lipids and L-α-PC. Ethidium/propidium fluorescence is studied by artificially imposing PMF in proteoliposomes. The major facilitator superfamily multidrug transporter LmrP from Lactococcuslactiscatalyses drug efflux using membrane potential and chemical proton gradient. LmrP contains catalytic carboxyl residues on the surface of a large interior chamber formed by transmembrane helices that enable the interactions with protons and cationic substrates. These residues co-localise together with polar and aromatic residues that is predicted to form two clusters. We have investigated the functional role of the catalytic carboxylates, by generating mutant proteins by removing one of the carboxyl residues in Cluster 1. This carboxyl residue was then relocated to six positions on the surface of the interior chamber, and tested for restoration of wildtype energetics. The reinsertion at positions towards Cluster 2 reinstated the membrane potential dependence of dye efflux. Our data discover a remarkable plasticity in proton interactions in LmrP, which in turn is due to the flexibility in the location of key residues that are involved in the proton/multidrug antiport.

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