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Structure and function of electrogenic sodium/proton antiporter membrane proteins[1]

OLIVER BECKSTEIN, Arizona State Univ

Sodium/proton antiporters are integral membrane proteins that are vital for cell homeostasis. In bacteria, they pump sodium ions out of the cell and enable survival in high-salt environments. In humans, they maintain cellular pH and their dysfunction is linked to a variety of complex diseases, including cancer, cardiovascular pathophysiology, and autism. Na^+/H^+ antiporters are secondary active transporters that utilize the electrochemical gradient of one ionic species to drive the energetically uphill transmembrane transport of the other species. They operate by the alternating access mechanism whereby the protein cycles between an outward facing and inward facing conformation to switch the exposure of substrate binding sites between the extracellular and the intracellular environment. We used a combination of molecular dynamics simulations with X-ray crystallography and functional measurements to address key questions about the transport mechanism in the two electrogenic bacterial antiporters NhaA and NapA, which both exchange 2 H^+ for 1 Na^+. In particular, the likely sodium and proton binding sites overlap, as predicted by a previously proposed competitive binding mechanism. A large "elevator-like" conformational transition moves the Na^+ binding site across the membrane, consistent with the alternating access mechanism. Based on simulations with varying protonation states of conserved ionizable residues and explicit constant pH MD simulations, we put forward a detailed hypothesis for the transport mechanism. Two protons are carried by a conserved aspartate and a conserved lysine residue. The Na+ binding site is formed by two conserved aspartate residues. The protonated lysine forms a salt bridge with the aspartate that does not carry a proton. Binding of Na^+ disrupts the salt bridge and facilitates the release of the proton from the lysine, thus maintaining the experimentally observed competitive binding mechanism. The binding site itself is translocated in a rigid fashion across the membrane, consistent with the alternating access mechanism. Functional transport measurements of mutants support the hypothesis that the Asp-Lys salt bridge is required for electrogenic transport.

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