Discovering the chloride conducting pathway of the CFTR channel using *in silico* methods

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Cystic fibrosis (CF) is a lethal monogenic disease caused by pathogenic variants of the CFTR/ABCC7 chloride channel, a member of the ATP Binding Cassette (ABC) protein superfamily. Most of the CF mutations affect protein folding and stability, leading to reduced apical anion conductance of epithelial cells. The recently published cryo-EM structures of full-length human and zebrafish CFTR provide a good model to gain insight into the relationship between structure and function of CFTR variants. While some of the structures were determined in the phosphorylated and ATP-bound active state, none of the static structures shows an open pathway for chloride permeation. Our goal is to characterize the CFTR structure and dynamics based on both experimental and homology models, using in silico methods. Therefore, we performed extensive molecular dynamics simulations to generate a conformational ensemble of the protein. Channel detecting algorithms were applied to identify conformations with open channel and the possible pathways were characterized. Since chloride ions entered the pathway in our equilibrium simulations without traversing the bottleneck region, we performed metadynamics simulations to describe the passage through this region. We defined the chloride pathway of CFTR at atomic resolution which suggests the existence of several intra- and extracellular entry sites. One of the extracellular exits includes hydrophobic lipid tails that may explain the lipid-dependency of CFTR function. In summary, our in silico study describes a potential chloride pathway based on a recent cryo-EM structure and it helps to understand the gating mechanism of the wild type and mutant CFTR proteins.

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