Revealing a New Conformational State in a Chloride/Proton Exchanger

"CLC" transporters are secondary active-transport membrane proteins that catalyze the transmembrane exchange of chloride (Cl^-) for protons (H^+). This exchange plays an essential role in proper cardiovascular, neuronal, muscular and epithelial functions. Several diseases arise from CLC defects, and several CLCs are therapeutic targets. For example, the CLC-7 transporter plays a critical role in bone remodeling through its contribution to acidification of osteoclasts thereby making it a therapeutic target for treating osteoporosis. In microbial pathogens, CLCs are virulence factors and are therefore potential drug targets to protect against bacterial food poisoning and fungal infections.

As secondary active transporters, CLC transporters can use the energy from Cl^- moving down its electrochemical gradient to pump protons *against* their electrochemical gradient, or vice versa. To achieve this remarkable disposition of energy, secondary active transporters such as the CLCs must tightly couple protein conformational change to substrate binding, unbinding and translocation. Since the dissipation of substrate gradients must be strictly avoided, a minimum of 3 different protein conformations is required: "outward-facing" (in which the external, but not internal, solution is accessible to ions), "occluded" (in which neither solution is accessible to ions), and "inward-facing" (in which the internal, but not external, solution is accessible). For all other transporters studied to date (including superfamilies with the MFS, LeuT, and NhaA folds) the conformational changes from outward-facing to occluded to inward-facing have been shown to involve global protein motions, including reorientation of helices or even entire domains. For the CLC transporters, in contrast, crystallographic studies have revealed only a single conformation, and models have posited that the transport mechanism may represent a different paradigm in which only localized side chain motions are involved.

Previously, the CLC transition from occluded to outward-facing states was proposed to involve only the rotation of a conserved glutamate side chain. This highly conserved residue, known as "Glu_{ex}", is located towards the extracellular side of the anion-permeation pathway, where it physically blocks anions from the extracellular solution (**Figure 1 A, B**). In the structure of a mutant in which Gln is used as a proxy for the protonated Glu_{ex}, the side chain is rotated away and appears to unblock the Cl^--permeation pathway. However, in this structure the pathway to the extracellular solution remains narrower than the diameter of Cl^- (**Figure 1B**). Therefore, the authors hypothesized that additional conformational changes occur to generate a true outward-facing state that allows free exchange of anions between the extracellular solution and the anion-permeation pathway.

To test this hypothesis, the interdisciplinary research team used a combination of NMR and DEER/EPR spectroscopy, chemical cross-linking, crystallography, and molecular dynamics (MD) simulations. The spectroscopic approaches revealed H^+-dependent conformational changes near the subunit interface of the homodimer, ~20 Å away from Glu_{ex}. To test the functional relevance of this motion, the scientists engineered a cysteine cross-link to constrict movement at this interface. Constriction of this motion via the cross-link correlated directly with reduced transport function.

The cross-linked protein was crystallized, and x-ray diffraction data were measured using Beam Line 12-2 at the Stanford Synchrotron Radiation Lightsource (SSRL). Since these membrane-protein crystals show wide variations in diffraction, the SAM (Stanford Automated Mounting system) robot was critical to identifying well-diffracting crystals. The structure of the cross-linked CLC, determined at 3.15 Å resolution, superimposes on the wild-type CLC structure with a Cα RMSD of 0.57 Å (**Figure 1C**). Together with the functional studies, this result demonstrates that the conformational state seen in all CLC structures to date is not merely a scaffold for the Glu_{ex} side-chain motion.

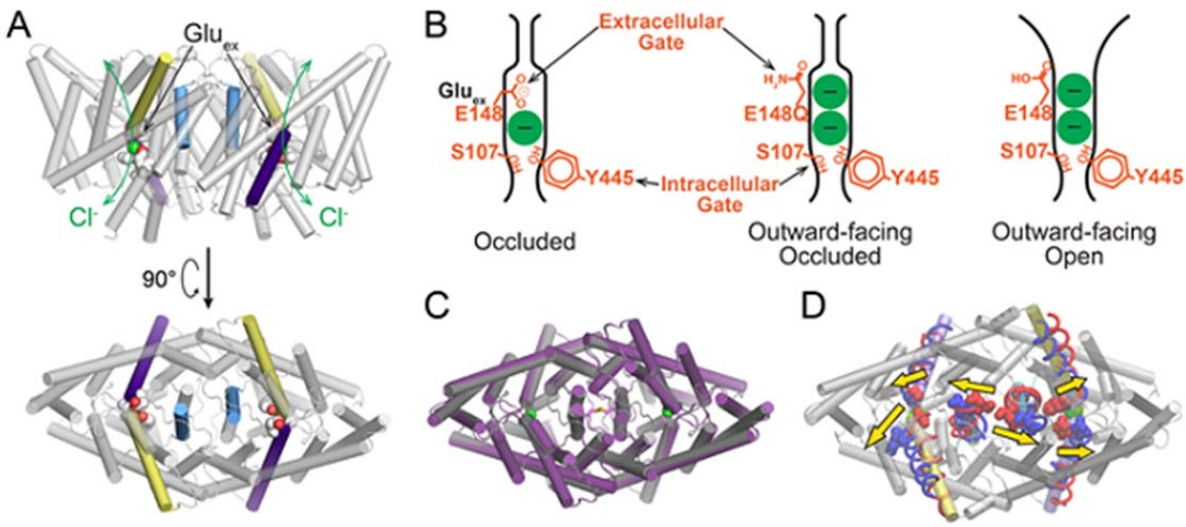

Figure 1. Structure and conformational change in CLC transporters. (**A**) Structure of CLC-ec1 (pdb: 1OTS), side view (*top panel*) and extracellular view (*bottom panel*). The conserved residue Glu_{ex} (one in each identical subunit) acts as a "gate" that blocks the Cl^--permeation pathway (green arrows) from the extracellular solution. The N-termini of helices F and N (purple and yellow) provide a positive electrostatic environment at the Cl^--permeation pathway. (**B**) Cartoon of the Cl^--binding region for WT (*left*) and Glu_{ex} → Gln mutant (*middle*). The right panel depicts the new conformational state revealed in this study. (**C**) The cross-linked D417C backbone determined in this study (PDB 5HD8, purple) superposes with WT (PDB 1OTS, grey) (Cα RMSD 0.57 Å). (**D**) Depiction of one of the extracellular gate-opening motions detected by analysis of the MD simulations. As the helices at the subunit interface (cyan) move away from one another, Helices F (purple) and N (yellow) move to open the extracellular gate. Initial and final positions of helices are shown in red and blue respectively.

To investigate the molecular basis of conformational change inhibited by the cross-link, extensive MD simulations were conducted on both the wild-type and cross-linked proteins. Analysis of the simulations revealed collective motions associated with opening of the extracellular gate and that these motions are dramatically dampened by the cross-link. An example of one of the extracellular gate-opening motions is shown in **Figure 1D**. Experimental tests of predictions arising from the simulation provided strong support for the model.

Together, these results describe a previously unidentified CLC "outward-facing open" conformational state. This finding provides essential new constraints for understanding the CLC

transport mechanism, overcoming the previous lack of data that precluded incorporation of global conformational change into models. This work also sets the stage for crystallographic structure-determination of the outward-facing open conformational state by guiding design of cross-linking studies to trap the state for crystallization. Such molecular details will be crucial for understanding the CLC transport mechanism and how it relates to those of canonical transporters.

Primary Citation

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Contacts

Merritt Maduke, Stanford University

Emad Tajkhorshid, University of Illinois at Urbana-Champaign

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